5-31-2019

The Use of Geospatial Modeling and Novel Diagnostics to Detect and Map Risk Factors of Soil-Transmitted Helminths in Feira de Santana, Brazil

Ryan Harry Avery
Louisiana State University and Agricultural and Mechanical College, ryanharryavery@gmail.com

THE USE OF GEOSPATIAL MODELING AND NOVEL DIAGNSOTICS TO DETECT AND MAP RISK FACTORS OF SOIL-TRANSMITTED HELMINTHS IN FEIRA DE SANTANA, BRAZIL

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Pathobiological Sciences

by
Ryan Harry Avery
B.S., State University of New York at Geneseo, May 2012
August 2019

This dissertation is dedicated to my family, especially my mother and father, without whom I would have never gotten to this point. I also dedicate this work to Maria Lauer, whose love, patience, and guidance have kept me together and allowed me to finish this.

"Tactically it may be required that we concentrate attack for amelioration and control on persons and communities most severely affected. Strategically, at the world level, we need to know of the enemy wherever he rears his head, in order that over-all planning omit no favorable opportunity for neutralizing him."

-Norman R. Stoll "This Wormy World"

Acknowledgments

First and foremost, I would like to thank God, my savior and my rock, for he has shepherded me through this whole process and given me the strength and courage to see this research through to the end.

To Maria Lauer, for the unending love, support, and understanding she has provided me with during this long dissertation process.

To my mother, father, Tyler, and Kylie, who encourage me to follow my passion and inspired me to persevere through adversity.

To my mentor Dr. John B. Malone, who has helped mold me into an inquisitive scientist and has ensured I never lost sight of the larger scientific picture.

To my Geospatial Health laboratory friends: Dr. Moara Martins, Dr. Cassan Pulaski, Dr. Elivelton Fonseca, Brooke Delcambre, and Trevor Atkins, thank you for all your guidance and contributions throughout the years. You have all made this work possible.

To my Graduate committee of Dr. Rebecca Christofferson, Dr. Thomas Klei, and Dr. James Miller, thank you for your advice and direction ensuring that I was able put forth my best effort and most complete work.

Table of Contents

Abbreviations and Acronyms

DALY: Disability-Adjusted Life Years

AUC: Area under the curve

ROC: Receiver operating characteristic

STH: soil-transmitted helminths

A. lumbricoides: Ascaris lumbricoides

T. trichiura: Trichuris trichiura

N. americanus: Necator americanus

A. duodenale: Ancylostoma duodenale

WHO: World Health Organization

NTD: Neglected Tropical Diseases

CDC: Center for Disease Control and Prevention

PCR: polymerase chain reaction

qPCR: quantitative polymerase chain reaction

WV2: WorldView-2

GE1: GeoEye-1

CDGN: Campo do Gado Novo

MOH: Ministry of Health

RS: Remote Sensing

GIS: Geographic Information Systems

Maxent: maximum entropy species distribution

Pre-SAC: pre-school aged children

SAC: school-aged children

VHR: very high-resolution

ENM: ecological niche model

UEFS: Universidade Estadual do Feira de Santana

UFBA: Universidade Federal da Bahia

Abstract

Soil-transmitted helminth (STH) infections impact billions of people worldwide. The traditional STH control approach is a morbidity control strategy implementing mass drug administration (MDA) programs targeting school-aged children (SAC). In Brazil, this control strategy has decreased STH prevalence to less than 20% in most of the country and providing an opportunity to transition from the morbidity control program and towards a surveillance and response system geared towards STH elimination. Surveillance and response systems geared towards elimination require the implementation higher accuracy diagnostics to detect infection in low-transmission communities, surveillance of entire households, high-resolution modeling at the household-habitat scale, and targeted treatment approaches tailored to specific communities. This study proposed to create a STH elimination surveillance and response system for the city of Feira de Santana, Brazil. Three communities were evaluated using three diagnostic techniques, entire households were sampled, and high resolution (30 m^2) and very high-resolution (VHR) (<5 m^2) satellite products were utilized to produce STH ecological niche models (ENMs). In a separate study, a zoonotic *Ascaris* case in the southern United States was examined. The comparison of the mini-FLOTAC, quantitative PCR (qPCR) and the Ministry of Health Kato-Katz thick smear diagnostic tests revealed that the qPCR diagnostic quantified a significantly greater number of hookworm eggs in stool samples than either the Kato-Katz or the NaCl mini-FLOTAC. Sampling revealed the need to test whole families, as the mean age of STH (29.03), hookworm (29.0), *Ascaris lumbricoides* (22.29), and *Trichuris trichiura* (19.2) infections demonstrated. The production of ENMs using the VHR satellites GeoEye-1 (GE1) and WorldView-2 (WV2), and the high-resolution satellite Landsat 8, demonstrated that GE1 provided either

superior or comparable model performed compared to WV2, and provided the resolution needed to effectively model STH niches at the household and its surrounding habitat. The differing STH prevalence and environmental risk factors between the 3 communities demonstrated the need to evaluate communities individually and craft targeted interventions. Genetic analysis in the case study determined that the cause of infection was zoonotic *Ascaris suum*. This study outlined steps towards implementing an STH elimination surveillance and response system for Fiera de Santana, Brazil.

Chapter 1. Introduction: A Paradigm Shift Toward an Elimination Strategy for Soil-Transmitted Helminths

1.1. Background

Soil-transmitted helminth (STH) infections impact over 1.5 billion people worldwide (Pullan et al. 2014). Several billion more people are at risk for STH infection, particularly those living in tropical environments and impoverished conditions (Bethony et al. 2006). The term STH is traditionally comprised of 4 different helminth species: the two hookworm species *Necator americanus* and *Ancylostoma duodenale*, the roundworm species *Ascaris lumbricoides*, and the whipworm species *Trichuris trichiura* (Jourdan et al. 2018). Although each species has different life cycles and biological requirements, they are normally grouped together due to their similar routes of infection, infection risk factors, and because they routinely co-infect human hosts. STHs are designated as a neglected tropical disease (NTD) by the World Health Organization (WHO). This classification identifies STH as a disease affecting people worldwide, but that has been historically underappreciated, under-examined, and underfunded in comparison to diseases such as malaria, HIV, and tuberculosis (Hotez et al. 2006).

The helminths comprising the STH group have similar life cycles. *A. lumbricoides* and *T. trichiura* are transmitted via a direct-fecal oral route and the two hookworm species are transmitted via a direct fecal-cutaneous route (Jourdan et al. 2018). All require time after human host defecation to develop into an infective stage. They persist and remain infective within the environment for varying amounts of time depending on species. For *A. lumbricoides* and *T. trichiura*, infective eggs can remain infective for months or even years in the environment due to their protective shells, whereas the free-living infective hookworm larvae (L3) can survive only a few weeks in the environment (Mudenda et al. 2012; Jourdan et al. 2018). Environmental

conditions play an important role for STHs development, as they require adequate moisture and higher temperatures to complete their life cycles (Bethony et al. 2006; Brooker et al. 2006; Mudenda et al. 2012). These environmental prerequisites, combined with lower socioeconomic status of inhabitants, are why STH endemic regions are mainly located in the tropical regions of the world (Bethony et al. 2006).

While STH infections are generally asymptomatic, they can also lead to severe morbidity and even mortality. This is particularly true for people with high worm burdens or vulnerable populations prone to serious morbidity, such as preschool-aged children (Pre-SAC) and school-aged children (SAC), women of child-bearing age, pregnant women, and lactating mothers (WHO 2012). For *A. lumbricoides* and *T. trichiura*, most heavy-intensity infections and morbidity is found in SAC (Anderson et al. 2013). However, for hookworm infections, adults harbor the highest worm burdens (Anderson et al. 2015). Infection risk is also linked to low socioeconomic status, with infections highest in those living in poverty with inadequate sanitation and hygiene. (Hotez et al. 2006; Hotez et al. 2009; Jourdan et al. 2018).

1.2. Shifting From Morbidity Control Towards Elimination

The traditional WHO-recommended control approach for STH infections is a morbidity control strategy implementing mass drug administration (MDA) programs targeting SAC (WHO 2012). This approach is utilized because of the high amount of morbidity and its corresponding detrimental growth outcomes in this age group, as well as the ease of access and administration of treatment at a centralized school location (Anderson et al. 2013). If possible, WHO also recommends treatment of the additional vulnerable populations of pre-SAC, women of child-bearing age, pregnant women, lactating mothers, and groups particularly exposed to infections

(i.e. tea-pickers) as these groups are also at high risk for serious morbidity and mortality. MDA treatment regimens consist of annual or bi-annual treatment with chemotherapeutic drugs. Annual treatment is recommended for areas with STH SAC prevalence of ≥20-<50%, and twice yearly treatments are recommended for areas with ≥50% SAC prevalence (WHO 2012). While these MDA control programs have been successful in highly endemic areas (Jourdan et al. 2018), they focus not on STH elimination and breaking the transmission cycle, but rather on the elimination of significant morbidity associated with STH infection. The goal of the MDA programs is to achieve <20% STH prevalence in SAC. After that benchmark is reached, treatment would shift from MDA to a case-by-case treatment approach (WHO 2012). The problem with this current morbidity control paradigm is that it neither interrupts transmission nor leads to elimination of infections within a community since a large portion of the population still harboring infections remain undiagnosed and untreated, contributing to the continued maintenance of STH transmission. This issue is of even greater importance in areas where hookworms cause the majority of STH infections, as adults harbor the majority of worms and act as reservoirs for reinfection (Anderson et al. 2013).

An emerging campaign to shift from a morbidity control approach and towards an NTD elimination approach has been advocated in recent years (Zhou et al. 2013; Tambo et al. 2014; Anderson et al. 2015; Bergquist et al. 2015; Brooker et al. 2015; Bergquist et al. 2017). The goal of this elimination approach would be to eliminate any STH infections within the entire population and interrupt transmission, similar to successful STH elimination programs previously enacted in the United States and Japan (Brooker et al. 2015), as opposed to traditional morbidity amelioration in vulnerable populations recommended by the WHO.

For this new elimination approach to be successful, a push towards creating and implementing surveillance and response systems geared towards elimination have been made (Zhou et al. 2013; Bergquist et al. 2015). A surveillance and response system is a well-designed public health scheme that systematically collects, analyzes, and interprets disease and health data, uses this data to identify risk areas and populations most impacted by the disease, provides public health personnel with appropriate disease interventions, and measures the efficiency and impact of these interventions (Zhou et al. 2013). The elimination of lymphatic filariasis in the People's Republic of China is an example of successful implementation of a surveillance and response system for elimination (Bergquist et al. 2015). To function properly, a surveillance and response system requires reliable data on spatiotemporal distribution, prevalence, incidence, and disease burden, with the end result of disease transmission interruption and eventual elimination of infections within an entire community (Tambo et al. 2014). To attain this, surveillance and response systems must incorporate more highly sensitive diagnostic testing to detect disease in low-transmission communities, and pair this with high-resolution modeling to evaluate disease risk and transmission within a given area.

In Brazil, there is no national STH control program due to budgetary constraints and the disease's lower national prevalence rates, and it is left to individual states and municipalities to enact control programs. The majority of Brazil is estimated to have an STH prevalence of <20% (Chammartin et al. 2014) and therefore considered low-risk areas of infection according to the WHO guidelines. Subsequently, many areas do not have any active control programs and rely on STH detection through passive health facility surveillance or by the Schistosomiasis Control Program, which leads to under-notification of STH infections (Martins-Melo et al. 2017). While

prevalence is <20%, STH infections continue to plague communities in Brazil (Almeida et al. 2012; Mariano et al. 2015; Faria et al. 2017; Monteiro et al. 2018), demonstrating that the current STH control approach is inadequate for elimination of the STH infections and transmission. We propose shifting the paradigm in the city of Feira de Santana, Bahia, in northeast Brazil, away from the current morbidity control approach that relies on passive surveillance and towards an active, targeted STH elimination approach. To achieve this paradigm shift, we created a surveillance and response system geared towards STH elimination in Feira de Santana.

1.3. Research Outline

Our study surveyed three representative communities of Feira de Santana, evaluating entire households regardless of age, with the objectives of 1) testing novel diagnostic techniques to determine if they provide improved STH egg detection rates compared to the currently used Kato-Katz thick smear technique and 2) evaluate the importance of testing entire households for STH instead of only vulnerable population (Chapter 3); we compared two very high-resolution (VHR) satellite products(<5 m^2), GeoEye-1 and WorldView-2, to 1) determine which provided superior predictive ecological niche models (ENMs) for STH and whether they could effectively model at the household-habitat level, 2) determine how VHR satellite ENMs (<5 m^2) compared to the coarser Landsat 8 satellite resolution (30 m^2), and 3) whether VHR satellites provided the resolution to detect STH environmental risk factor differences based on community type (Chapter 4). In a separate study, we evaluated a case of zoonotic *Ascaris suum* in the southern United States and discuss why continued STH vigilance is important, even in areas that have previously eliminated human to human transmission (Chapter 5).

The combination of higher-accuracy diagnostics, paired with high-resolution household-habitat ENMs allowed for the creation of a STH surveillance and response system for Fiera de Santana, Brazil. The subsequent public health policy recommendations based on this surveillance and response system, and the next phases of the elimination plan, are described in the conclusion (Chapter 6).

1.4. References

Almeida PHA de, Santana PCS, Silva AV da. 2012. Prevalência de protozoários e helmintos entéricos em residentes de são cristóvão, feira de santana, bahia, brasil. Arq. Ciênc. Saúde UNIPAR 16:61–66.

Anderson RM, Truscott JE, Pullan RL, Brooker SJ, Hollingsworth TD. 2013. How Effective Is School-Based Deworming for the Community-Wide Control of Soil-Transmitted Helminths? PLoS Negl. Trop. Dis. 7:e2027. doi:10.1371/journal.pntd.0002027.

Anderson RM, Turner HC, Truscott JE, Hollingsworth TD, Brooker SJ. 2015. Should the Goal for the Treatment of Soil Transmitted Helminth (STH) Infections Be Changed from Morbidity Control in Children to Community-Wide Transmission Elimination? Aksoy S, editor. PLoS Negl. Trop. Dis. 9:e0003897. doi:10.1371/journal.pntd.0003897.

Bergquist R, Yang GJ, Knopp S, Utzinger J, Tanner M. 2015. Surveillance and response: Tools and approaches for the elimination stage of neglected tropical diseases. Acta Trop. 141:229–234. doi:10.1016/j.actatropica.2014.09.017.

Bergquist R, Zhou XN, Rollinson D, Reinhard-Rupp J, Klohe K. 2017. Elimination of schistosomiasis: The tools required. Infect. Dis. Poverty 6:158. doi:10.1186/s40249-017-0370-7.

Bethony J, Brooker S, Albonico M, Geiger SM, Loukas A, Diemert D, Hotez PJ. 2006. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. Lancet 367:1521–1532. doi:10.1016/S0140-6736(06)68653-4.

Brooker S, Clements ACA, Bundy DAP. 2006. Global Epidemiology, Ecology and Control of Soil-Transmitted Helminth Infections. Adv. Parasitol. 62:221–261. doi:10.1016/S0065-308X(05)62007-6.

Brooker SJ, Nikolay B, Balabanova D, Pullan RL. 2015. Global feasibility assessment of interrupting the transmission of soil-transmitted helminths: A statistical modelling study. Lancet Infect. Dis. 15:941–950. doi:10.1016/S1473-3099(15)70042-3.

Chammartin F, Guimarães LH, Scholte RGC, Bavia ME, Utzinger J, Vounatsou P. 2014. Spatio-temporal distribution of soil-transmitted helminth infections in Brazil. Parasites and

Vectors 7:440. doi:10.1186/1756-3305-7-440.

Faria CP, Zanini GM, Dias GS, da Silva S, de Freitas MB, Almendra R, Santana P, Sousa M do C. 2017. Geospatial distribution of intestinal parasitic infections in Rio de Janeiro (Brazil) and its association with social determinants. Raso G, editor. PLoS Negl. Trop. Dis. 11:e0005445. doi:10.1371/journal.pntd.0005445.

Hotez P, Ottesen E, Fenwick A, Molyneux D. 2006. The neglected tropical diseases: The ancient afflictions of stigma and poverty and the prospects for their control and elimination. In: Advances in Experimental Medicine and Biology. Vol. 582. Springer US. p. 23–33.

Hotez PJ, Fenwick A, Savioli L, Molyneux DH. 2009. Rescuing the bottom billion through control of neglected tropical diseases. Lancet 373:1570–1575. doi:10.1016/s0140-6736(09)60233-6.

Jourdan PM, Lamberton PHL, Fenwick A, Addiss DG. 2018. Soil-transmitted helminth infections. Lancet 391:252–265. doi:10.1016/S0140-6736(17)31930-X.

Mariano APM, Santos EN, Santos TN dos, Mota TN, Silva JA da, Carvalho SMS, Silva MF da. 2015. Parasites in South Bahia: Focus on Giardiasis and Ascariasis among Preschoolers of Itabuna. Int. J. Heal. Sci. 3. doi:10.15640/ijhs.v3n1a4.

Martins-Melo FR, Ramos AN, Alencar CH, Lima MS, Heukelbach J. 2017. Epidemiology of soil-transmitted helminthiases-related mortality in Brazil. Parasitology 144:669–679. doi:10.1017/S0031182016002341.

Monteiro KJL, Reis ERC Dos, Nunes BC, Jaeger LH, Calegar DA, Santos JP Dos, Maia A de O, Xavier SC das C, Bóia MN, Carvalho-Costa FA, et al. 2018. Focal persistence of soil-transmitted helminthiases in impoverished areas in the State of Piaui, Northeastern Brazil. Rev. Inst. Med. Trop. Sao Paulo 60:e24. doi:10.1590/s1678-9946201860024.

Mudenda NB, Malone JB, Kearney MT, Mischler PD, Del P, Nieto M, McCarroll JC, Vounatsou P. 2012. Modelling the ecological niche of hookworm in brazil based on climate. Geospat. Health 6.

Pullan RL, Smith JL, Jasrasaria R, Brooker SJ. 2014. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. Parasit. Vectors 7:37. doi:10.1186/1756-3305-7-37.

Tambo E, Ai L, Zhou X, Chen J-H, Hu W, Bergquist R, Guo J-G, Utzinger J, Tanner M, Zhou X-N. 2014. Surveillance-response systems: the key to elimination of tropical diseases. Infect. Dis. poverty 3:17. doi:10.1186/2049-9957-3-17.

WHO. 2012. Soil-transmitted helminthiases : eliminating as public health problem soil-transmitted helminthiases in children : progress report 2001-2010 and strategic plan 2011-2020. Geneva: World Health Organization.

Zhou XN, Bergquist R, Tanner M. 2013. Elimination of tropical disease through surveillance and

response. Infect. Dis. Poverty 2:1–5. doi:10.1186/2049-9957-2-1.

Chapter 2. Literature Review

2.1. Soil-Transmitted Helminths

Soil-transmitted helminth (STH) infections are a global public health problem, infecting over 1.5 billion people, with billions more at risk. They occur predominantly in warm, tropical climates (de Silva et al. 2003; Bethony et al. 2006; Brooker 2010; Pullan et al. 2014). STH infections are caused primarily by the whipworm *Trichuris trichiura*, the roundworm *Ascaris lumbricoides*, and two species of hookworm, *Necator americanus* and *Ancylostoma duodenale* (Bethony et al. 2006; Brooker et al. 2006; Brooker 2010). All three parasite types belong to the Kingdom Animalia, Phylum Nematoda (*Latin for thread-like*). Global prevalence estimates for 2010 were 819 million for *A. lumbricoides*, 464.6 million for *T. trichiura*, and 438.9 million for hookworm (Pullan et al. 2014). The World Health Organization (WHO) considers STH to be a neglected tropical diseases (NTD), a category encompassing diseases historically underfunded, under-researched, and overlooked due to the focus on other diseases such as malaria, tuberculosis, and HIV that exist in those same areas (Hotez et al. 2006; WHO 2012).

Many STH infections are asymptomatic, but can cause severe morbidity and even mortality, especially in infections with heavier worm burdens. Infections have the largest impact on already vulnerable populations, such as preschool-aged children (Pre-SAC) and school-aged children (SAC) (Figure 2.1.), women of child-bearing age, pregnant women, and lactating mothers (WHO 2012). Infection risk is highest in those living in poverty, and that have inadequate sanitation and hygiene (Hotez et al. 2006; Hotez et al. 2009; Jourdan et al. 2018). These infections compound this poverty in vulnerable populations through reduced worker productivity and subsequently decreased salary, social stigma, and life-long disabilities (Hotez et al. 2006; Hotez

et al. 2009). STH infection intensity and subsequent morbidity are highest in pre-SAC and SAC, and therefore

WHO control programs are geared towards treating these groups (WHO 2012; Turner et al. 2015). If feasible, additional at-risk populations of pregnant women, lactating mothers, and women of child-bearing age are also included in these programs. The primary goal of these WHO mass drug administration (MDA) programs is to combat morbidity associated with STH infection, not to eliminate the disease itself (WHO 2012). The WHO definition of elimination of STHs as a public health problem is when prevalence in SAC is <20%. At that point, STH treatment would be on a case-by-case basis.

Morbidity issues can include impaired cognitive and physical growth, impaired physical fitness, and decreased school performance, gastrointestinal pain, nutrient malabsorption, and anemia (Bethony et al. 2006; Brooker et al. 2008; Dold and Holland 2011; Kassebaum et al. 2014; Blouin et al. 2018; Pabalan et al. 2018). STHs can cause mortality, especially *A. lumbricoides* infections, by creating deadly acute complications due to adult worm migration and aggregation (de Silva et al. 1997; Pullan et al. 2014). The much greater impact of STH infections is due to the serious morbidity they cause throughout life, especially the impact on young children and pregnant women. To accurately assess this morbidity and its effects on an infected population, STH impact is assessed in terms of disability-adjusted life years (DALYs) (Murray 1994; Bethony et al. 2006; Murray, Vos, et al. 2012). The DALY equation takes into account the time lived with disability, the severity of disability, the time lost due to premature death, and the value of life lived at various ages (Murray 1994). The annual DALYs for STH has been calculated as 5.18 million in 2010, with *A. lumbricoides* having 1.31 million, *T. trichiura* having 0.64 million, and hookworm having 3.23

million DALYs (Murray, Vos, et al. 2012). As a frame of reference the 2010 Global Burden of Disease study by Murray, Vos, et al. (2012) calculated that the annual DALY for leishmaniasis as 3.32 million and malaria as 82.69 million. While not at the DALY measure of malaria, STH infection ranked first among the NTDs in DALY total with 5.18 million. This displays how impactful STH infection is globally and the amount of morbidity that the disease causes.

Measuring the intensity of STH infection within an individual and throughout the community is important as it is a main indicator of infection morbidity (WHO 2011). To measure intensity of STH infection, fecal egg counts (FEC) of each type of parasite are obtained and reported as eggs per gram (EPG). EPG counts are used as an indirect measure of worm burden (WHO 2011), and a proxy for intensity of infection. Table 2.1. illustrates the three classes of infection intensity for each STH: for *A. lumbricoides* light-intensity 1-4,999 EPG, moderate-intensity 5,000-49,999 EPG, heavy-intensity ≥50,000 EPG; for *T. trichiura* light-intensity 1-999 EPG, moderate-intensity 1,000-9,999 EPG, heavy-intensity ≥10,000 EPG; and for hookworm light-intensity 1-1,999 EPG moderate-intensity 2,000-3,999 EPG, heavy-intensity ≥4,000 EPG. Accurately assessing intensity, especially in heavy-intensity infections, is important for control programs since their first objective is to reduce the total number of heavy-intensity individuals (WHO 2011). Treating these high-infection intensity individuals is important both because they are most likely to suffer from sever morbidity and because they are the main drivers of STH transmission within the community. STH infection has a worldwide distribution but is endemic in areas with sub-tropical and tropical climates. They are found predominantly in the tropical climes because their life cycles require adequate moisture and higher temperatures to properly develop (Bethony et al. 2006; Brooker et al. 2006). In addition, the impoverished communities found

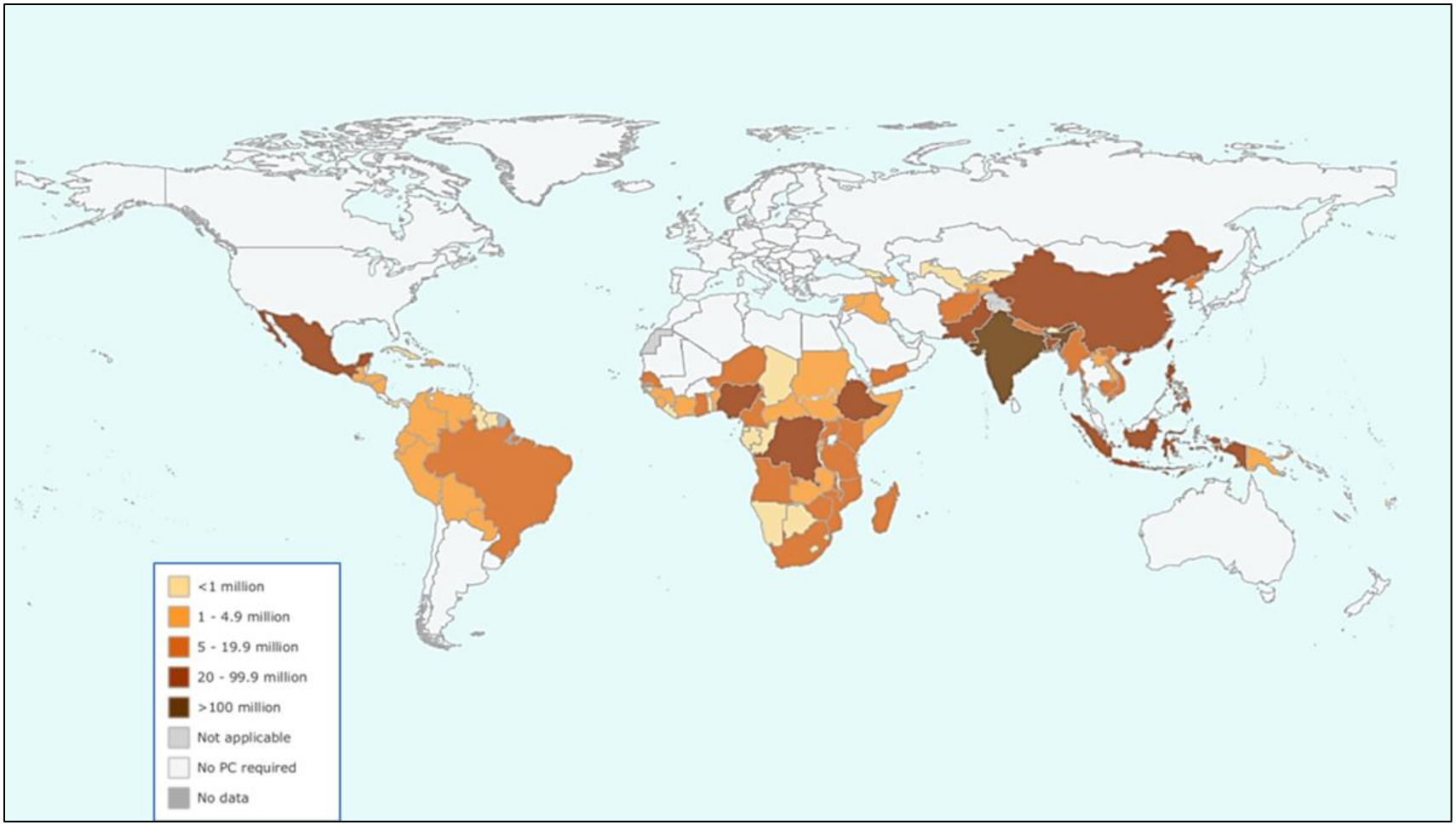

Figure 2.1. Global map displaying number of pre-school age children and school age children requiring chemotherapy for STH infection in 2017. (Adapted from World Health Organization Interactive Maps, 2019. http://apps.who.int/neglected_diseases/ntddata/sth/sth.html)

throughout many of these tropical regions increases the risk of STH infection and continuing transmission.

Table 2.1. Classes of intensity of individual infection for soil-transmitted helminths and schistosomes based on the WHO Expert Committee Report (WHO 2002). (WHO 2011)

Parasite	Light-intensity infections[b]	Moderate-intensity infections[b]	Heavy-intensity infections[b]
A. lumbricoides	1–4 999 epg	5 000–49 999 epg	≥50 000 epg
T. trichiura	1–999 epg	1 000–9 999 epg	≥10 000 epg
Hookworms	1–1 999 epg	2 000–3 999 epg	≥4 000 epg
S. mansoni	1–99 epg	100–399 epg	≥400 epg
S. haematobium	1–50 eggs/10 ml of urine		>50 eggs/10 ml of urine (or visible haematuria)

[a] WHO, 2002.
[b] epg = eggs per gram of faeces.

2.1.1. STH Infections in Brazil

STH infections have a large impact on the continent of South America, particularly in countries such as Brazil (Murray et al. 2012). The 2010 estimated number of those infected with STHs in tropical Latin America (Murray et al. 2012), which encompasses the majority of Brazil with a total population of 205.4 million, was 48.5 million (Pullan et al. 2014). Hookworm infections affected 11 million (5.4% prevalence), *A. lumbricoides* 24.5 million (11.9% prevalence), and *T. trichiura* 13 million (6.4%) of the STH infection total. This translates to 124,199 years lived with disability (YLDs) for hookworm, 21, 865 YLDs for *A. lumbricoides*, and 11,120 YLDs for *T. trichiura* (Pullan et al. 2014). Brazil had a national STH control program until 2005, when it was discontinued (WHO 2012), and now each state and municipality is responsible for implementation of STH control programs.

Currently, the majority of surveillance is conducted passively, through either health institutions or the national Schistosomiasis control program (Brazilian Ministry of Health 2012;

Chammartin et al. 2014; Martins-Melo et al. 2017). This under-notification and subsequent lack of adequate treatment has a disproportionate effect on children and people at the bottom of the socioeconomic ladder (Bethony et al. 2006).

While normally these helminths are grouped together as STHs, they vary markedly from each other biologically and this must be considered when treating humans and creating control programs. Understanding the biological and ecological differences between species is important when attempting to combat STH infection in communities (Table 2.2.).

Table 2.2. The population parameters, life cycle requirements, life-spans, and reproductive numbers (secondary infections caused by a single primary infection) of *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm. (Brooker et al. 2006)

	A. lumbricoides	*T. trichiura*	Hookworm
Infective stage	Ova	Ova	Larvae
Egg production (eggs/female worm/day)[a]	10 000–200 000	2000–20 000	3000–20 000
Life expectancy of free-living infective stages[a]	28–84 days	10–30 days	3–10 days
Adult life span[b]	1–2 years	1–2 years	3–4 years
Pre-patency (adult development to sexual maturity)[b]	50–80 days	50–84 days	28–50 days
Larvae development time to infective stage[c]	8–37 days	20–100 days	2–14 days
Max. temp. of viable development[c]	35–39°C	37–39°C	40°C
Basic reproductive number[b]	1–5	4–6	2–3

[a]Data taken from Anderson (1982), Bundy and Cooper (1989) and Crompton (2001).
[b]Data taken from Anderson and May (1991).
[c]Data on *A. lumbricoides* (Seamster (1950); *T. suis* (Beer, 1976); hookworm (Nwosu, 1978; Smith and Schad, 1989)).

2.1.2. *Ascaris lumbricoides*

A. lumbricoides, informally known as the common roundworm (GenBank common name), is a gastrointestinal nematode that occurs in humans worldwide. It is classified phylogenetically in the Class Secernentea, Order Ascaridida, and Family Ascarididae (Figure 2.2.). *A lumbricoides* is transmitted via a direct fecal-oral cycle and is closely linked with poverty and poor sanitation.

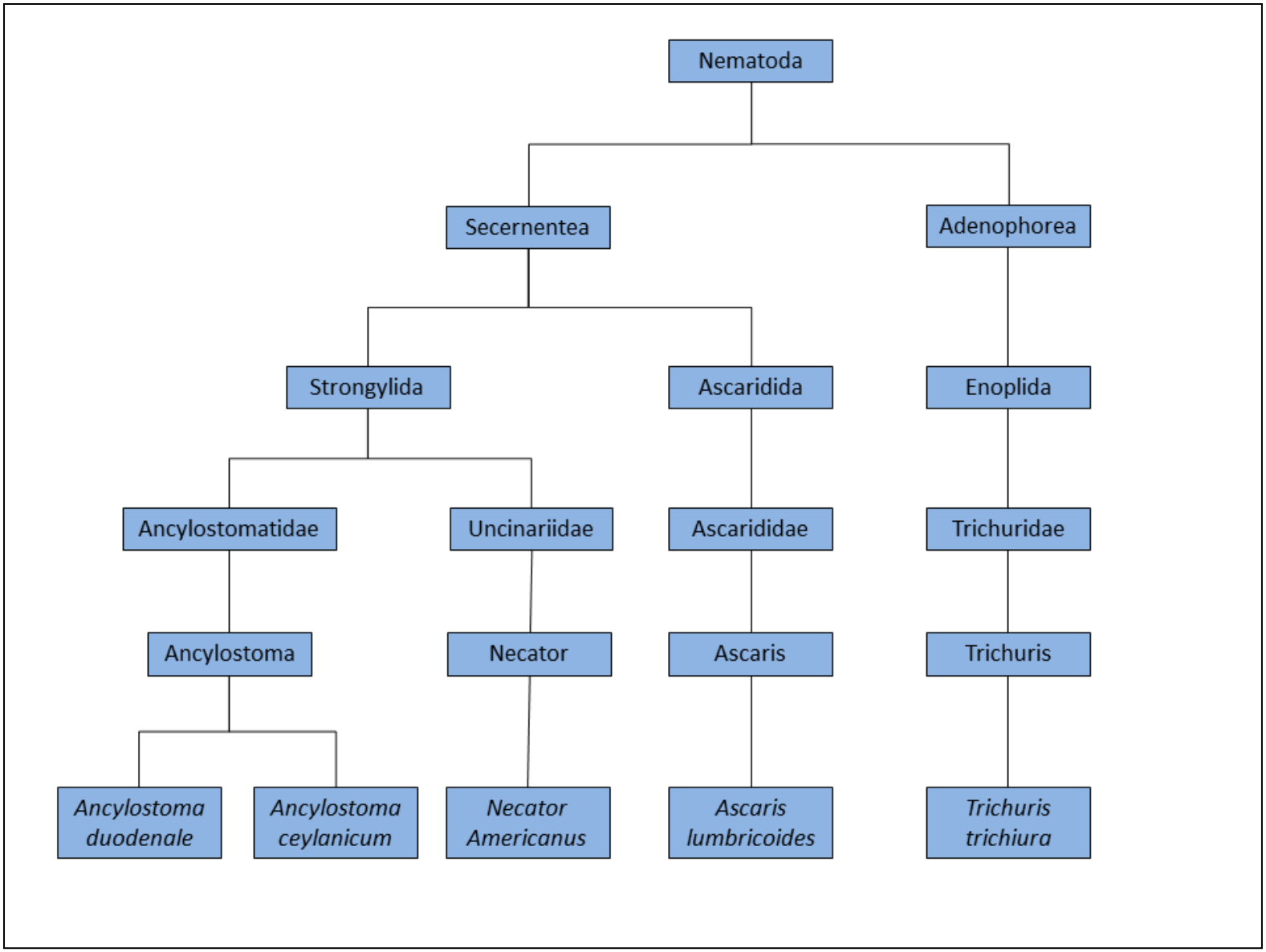

Figure 2.2. A taxonomic diagram displaying the relationship between hookworm species, *Ascaris lumbricoides*, and *Trichuris trichiura.*

Often asymptomatic, *A. lumbricoides* infection can cause intestinal pain, malnutrition, and impaired cognitive and physical development (Bethony et al. 2006). In the lung migratory phase, larval *A. lumbricoides* may cause severe pulmonary eosinophilic pneumonitis known as Loeffler syndrome. While *A. lumbricoides* is considered a human pathogen and *A. suum* a swine pathogen, *A. suum* has been reported in humans, *A. lumbricoides* has been reported in swine, and cross-hybridization between the two has been demonstrated (Nejsum et al. 2012). While most infections remain asymptomatic, heavy-intensity infections can lead to severe morbidity and even death (Dold and Holland 2011). Pullan et al. (2014), calculated that the number of deaths caused by *A. lumbricoides* in 2010 was 2,824 worldwide. The larger issue with *A. lumbricoides* infections is the serious morbidity it imposes, particularly on those most vulnerable. Infections can contribute to nutrient deficiency such as vitamin A deficiency, especially in children (de Gier et al. 2016).

The life cycle of *A. lumbricoides* (Figure 2.3.) consists of adult females releasing eggs in the small intestine that are then passed with the feces. Eggs remain in the fecal matter and soil, where they embryonate into infective larvae after ~2 weeks at ~30°C. The time for larval development is highly dependent on the environmental conditions, with moist, warm, shaded soil the optimal conditions. The mamillated egg coat is extremely adhesive and easily adheres to unwashed food and hands. Infective eggs are ingested and the larvae hatch and invade the intestinal mucosa. Larva are carried via the portal and then systemic circulatory systems to the lungs, where they continue to mature for 10-14 days. Larvae then penetrate the alveolar walls, ascend the bronchi, and are subsequently swallowed. Once they reach the small intestine, they finish their development into adult worms.The entire time from ingestion of eggs to a gravid

female is between 2-3 months. An adult *A. lumbricoides* lifespan ranges from 1-2 years (O'Lorcain and Holland 2002).

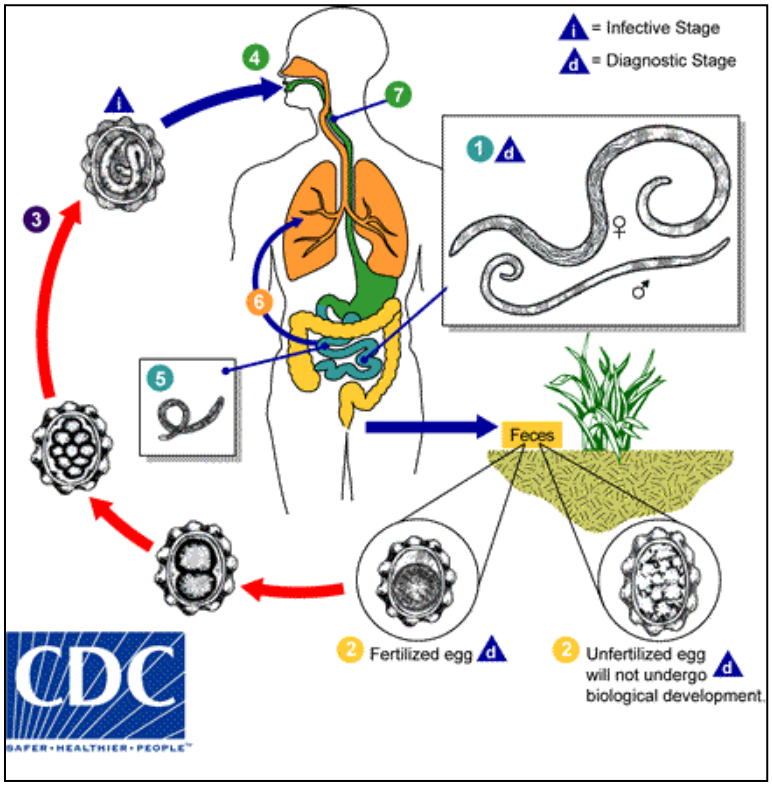

Figure 2.3. The life cycle of *Ascaris lumbricoides* in a human host
(Source: https://www.cdc.gov/dpdx/ascariasis/index.html)

2.1.3. Hookworm (*Necator americanus*, *Ancylostoma duodenale*)

Human hookworm disease is primarily caused by two species, *N. americanus* and *A. duodenale*. *A. duodenale* belongs to Class Secernentea, subclass Rhabditia, Order Strongylida, Family Ancylostomatidae. *A. duodenale* was first discovered and documented by the Italian physician Angelo Dubini in 1838 (The Rockefeller Foundation-International Health Board 1922). *N. americanus* belongs to Class Secernentea, subclass Rhabditia, Order Strongylida, Family Uncinariidae (Figure 2.2.). *N. americanus* was first recognized and described as a new separate species of hookworm from *A. duodenale* by Dr. Charles Wardell Stiles (1902a; 1902b). While colloquially called "the American hookworm", *N. americanus* is an old-world hookworm most

likely imported from Africa via the slave trade. It was the first human hookworm genome to be completely characterized (Tang et al. 2014), and is the target of an ongoing vaccination project due to the intense anemia it causes (Hotez et al. 2013; Brelsford et al. 2017; Diemert et al. 2017). *Ancylostoma ceylanicum* is a third species of hookworm that infects both canids and humans, but is only found in Asia and Australian and is of only minor significance (Bonne 1937; Carroll and Grove 1986; Bethony et al. 2006; Smout et al. 2017; O'Connell et al. 2018).

Hookworms have a direct life cycle where gravid adult females shed eggs in the feces (Figure 2.4.). Eggs hatch within 1-2 days and grow in the fecal/soil environment for 5-10 days. During this time, they molt twice to become infective third-stage (filiform) larvae (L3). These L3 can survive for up to 4 weeks while waiting for a human host. When they contact human skin, generally the foot and ankle area, they penetrate and enter blood vessels. They are then carried to the heart and lungs, where they penetrate pulmonary alveoli, travel up to the pharynx and are swallowed. They continue to travel through the gastrointestinal (GI) system until they arrive in the proximal small intestine, where they mature into adults and repeat the cycle.

Depending on species, a female can produce anywhere from 9,000-10,000 (*N. americanus*) to 25,000-30,000 eggs per day. Adults can survive in the proximal small intestine for 5-7 years. Hookworms cannot survive out of the host for very long. This contrasts with both *Ascaris lumbricoides* and *Trichuris trichiura*, which are adapted to stay viable for long periods outside of the host, but live as adult worms in the host for only 1-2 years (Bethony et al. 2006).

Hookworm infection is the most taxing on the infected population due to its blood feeding activities that lead to anemia (Crompton 2000). Infection is the leading cause of anemia in parts of Africa and Oceania (Hotez et al. 2016). Between 3,000-65,000 annual deaths are attributed to

hookworm infections. The larger issue is their massive impact in terms of morbidity. Hookworm infection is estimated to cause the loss of between 1.8-22.1 million DALYs annually (Hotez et al. 2009).

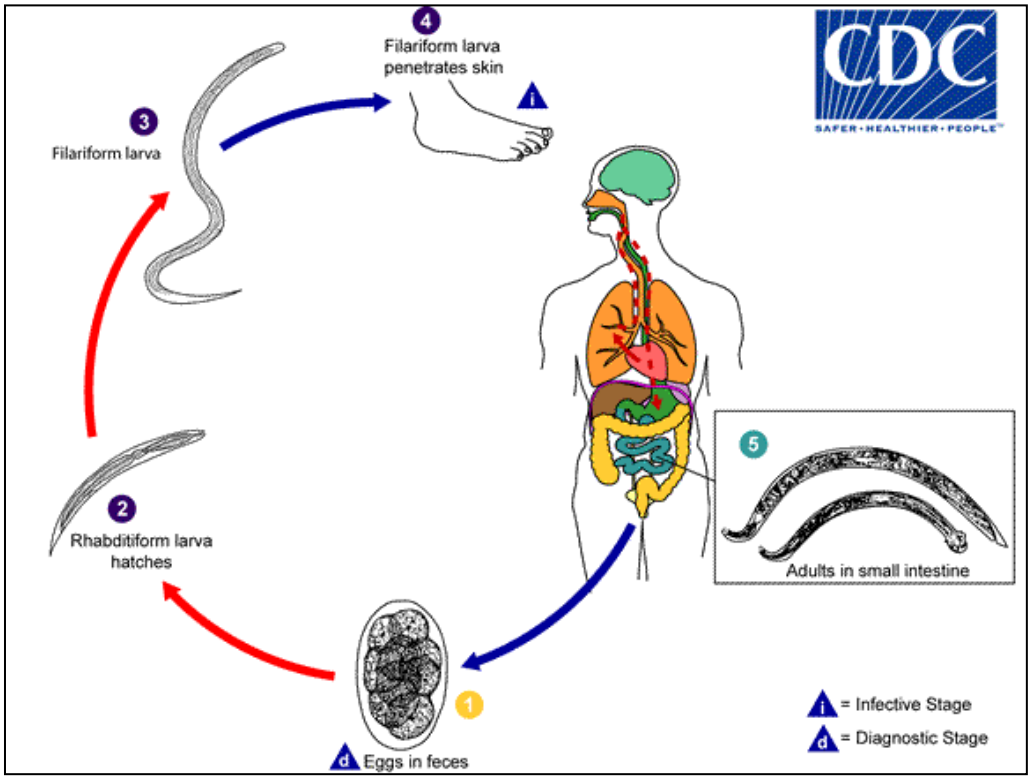

Figure 2.4. The life cycle of the human hookworm in a human host.
(Source: https://www.cdc.gov/dpdx/hookworm/index.html)

2.1.4. *Trichuris trichiura*

Trichuriasis in humans is caused by the nematode *T. trichiura*. *T. trichiura* belongs to Class Adenophorea, Order Enoplida, Family Trichuridae (Figure 2.2.). Transmission of *T. trichiura* is a direct fecal-oral transmission cycle, with eggs passed with the feces (Figure 2.5.). Eggs stay on the ground and continue to mature for 15-30 days before growing into an infective larva within the egg. Infective eggs are ingested and hatch, maturing as they move along the GI tract until reaching the large intestine, where they reside as adults. Light infections of *T. trichiura* are usually asymptomatic, but like hookworm and *A. lumbricoides*, heavier intensity infections can cause

serious morbidity, including diarrhea, malnutrition, anemia, and growth retardation (Manz et al. 2017). The worldwide toll of *T. trichiura* infection has been estimated to cause the loss of between 1.8-22.1 million DALYs annually (Hotez et al. 2009). *T. trichiura* infections are the most difficult of the three STH parasites to treat, as many of the available drugs are only partially effective against infection and require repeated days of treatment for efficacy (Jourdan et al. 2018).

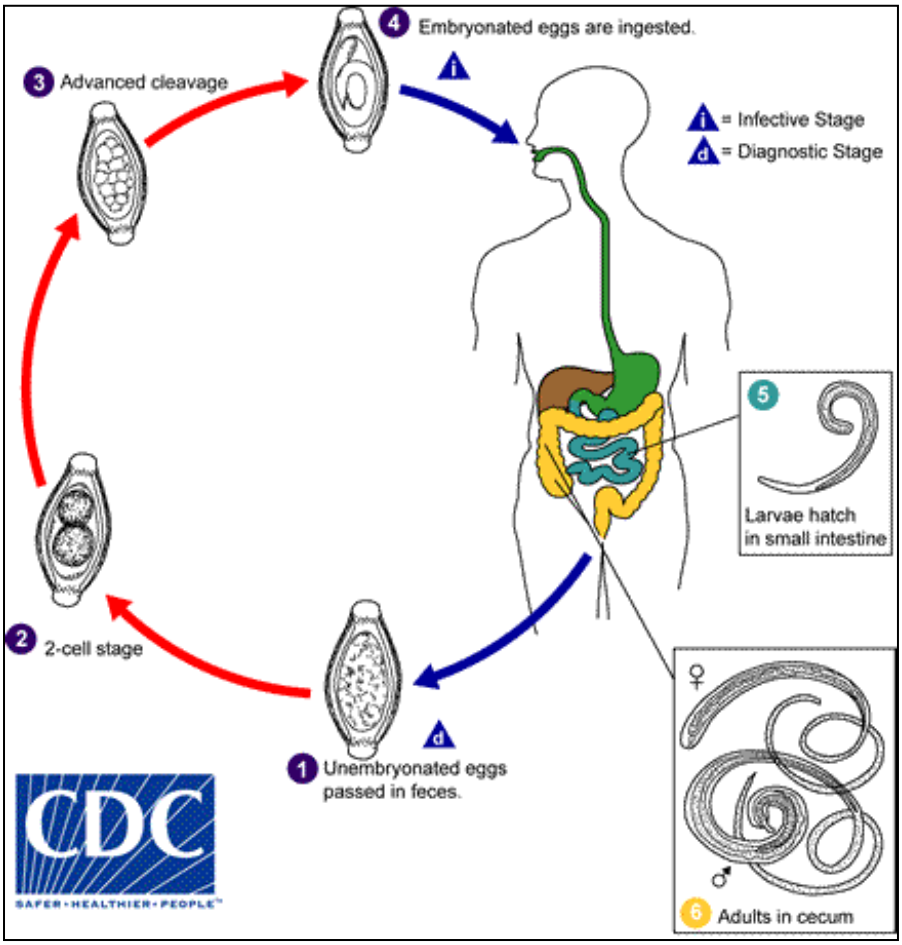

Figure 2.5. The life cycle of the *Trichuris trichiura* in a human host.
(Source: https://www.cdc.gov/dpdx/trichuriasis/index.html)

2.2. Diagnostic Techniques, Treatment, and Control

Definitive diagnosis of STH infection throughout the world still mainly relies on light microscopy techniques. The WHO recommended method for public health detection is the Kato-

Katz thick smear (WHO 1991; WHO 1994; WHO 2011). The Kato-Katz thick smear was first described by Kato and Miura (1954), then improved upon by others, including Martin and Beaver (1968), Chaia et al. (1968), and Katz et al. (1972). The technique is simple and straightforward to run, requires very little fecal sample, minimal equipment, inexpensive, and training can be finished in half a day (Kongs et al. 2001; WHO 2004; Speich et al. 2010). Traditionally, various other light microscopy techniques have been used to quantify the number of eggs in a given amount of fecal sample. The spontaneous sedimentation technique (Lutz 1919; Hoffman et al. 1934) is still used in many areas of the world due to its ease of use and the ability to examine for protozoa as well. The McMaster egg counting method (Levecke et al. 2011) is a flotation technique commonly used in veterinary parasitology that has been evaluated for use in human infections. Newer diagnostic tests such as the FLOTAC (Knopp et al. 2009), and mini-FLOTAC (B.D. Barda et al. 2013) have been created to attempt to improve upon the Kato-Katz thick smear technique while still allowing affordability and ease of use in field settings (B. Barda et al. 2013). Several multiplex PCR tests for STHs (Mejia et al. 2013; McCarthy et al. 2017; Cunningham et al. 2018) have been recently created and are being field tested and optimized. The loop-mediated isothermal amplification method, is a nucleic acid test that can be implemented without the use of a thermocycler (Amoah et al. 2017).

Treatment of STH infection generally employs one of the benzimidazoles, and treatment length is generally once (Jourdan et al. 2018), thus making MDA programs easier to employ. Albendazole or mebendazole is given orally (Table 2.3.) and is effective against *A. lumbricoides* and hookworm. Due to the lack of efficacy against *T. trichiura*, a 3 consecutive day treatment

regimen should be implemented (Table 2.3.). Alternatively, ivermectin and pyrantel embonate have been used as treatments for *A. lumbricoides* and *T. trichiura* (Jourdan et al. 2018).

Table 2.3. Recommended first and alternative choices and dose description for oral chemotherapeutic treatments to treat infections with *Ascaris lumbricoides*, *Trichuris trichiura*, and *Necator americanus* and *Ancylostoma duodenale* hookworm infections.

	First Choice Treatment	Alternative Treatment
A. lumbricoides	Albendazole:1x 400 mg Mebendazole:1x 500 mg Mebendazole:2x 100 mg daily for 3 days	Ivermectin:1x 150-200 µg/kg
T. trichiura	Albendazole:1x 400 mg daily for 3 days Mebendazole:1x 500 mg daily for 3 days Mebendazole:2x 100 mg daily for 3 days	Ivermectin:1x 200 µg/kg daily for 3 days Pyrantel embonate:11 mg/kg base (maximum of 1 g) 1x daily for 3 days
Hookworm (*N. americanus*, *A. duodenale*)	Albendazole:1x 400 mg Mebendazole:1x 500 mg Mebendazole:2x 100 mg daily for 3 days	None

2.3. Geospatial Technologies

A geographic information system (GIS) is a computer system that allows for the combination and manipulation of spatial and descriptive data for mapping, modeling, and analysis (Brooker et al. 2002). The term GIS was first introduced into the field of geography by Tomlinson (1969). Remote sensing (RS), is the science of data acquisition of an entity of interest without coming into contact with it (Jensen 2007). This can be achieved with aircraft and/or satellite sources that record information from the electromagnetic spectrum. The term originated in the 1960s by Evelyn L. Pruitt to describe the expansion of data acquisition from the visible and near-infrared spectrum into additional parts of the electromagnetic spectrum (Jensen 2007). Together, RS and GIS have since combined to allow geographers and other scientists to acquire and process a broad array of spatial and temporal information to observe and map the earth. GIS and RS have also become important aspects of assessing public health and epidemiology, and have been used with increasing frequency to model STH infection across the

globe (Chammartin et al.; Brooker et al. 2006; Mudenda et al. 2012; Chammartin et al. 2014; Soares Magalhães et al. 2015).

2.3.1. Ecological Niche Models and Maximum Entropy Species Distribution Modeling

Ecological niche models (ENMs) are a group of methods that create models that predict a species habitat suitability using species occurrence data in conjunction with environmental data from the occurrence locations (Warren and Seifert 2011). ENMs can be used for estimating relative habitat suitability of a species, either known to occur in that area or not known to occur in a given area. In addition, ENMs allow for analysis of the temporal change of habitat suitability due to temporal changes in the environment, and can be used to estimate a species niche (Warren and Seifert 2011).

One of the most often employed tools for producing ENMs is the maximum entropy species distribution (Maxent) modelling tool (Phillips et al. 2006). Maxent allows for the use of occurrence only data to create ENMs for a species to accurately measure their geographic distribution and ecological niche (Phillips et al. 2006). Maxent estimates the species distribution in a given space through the creation of numerous species distributions to determine which has the maximum amount of entropy, or is closest to uniform, while constraining the model using environmental values at occurrence points (Phillips et al. 2006; Phillips et al. 2017). The model's underlying maximum entropy principle signifies that out of the many distribution models created that satisfy these constraints, the one with maximum entropy, or closest to uniform, be chosen.

2.4. Surveillance and Response Systems

The move towards elimination is feasible in areas of lower endemicity and where there is proper government funding and desire for elimination. To enable a paradigm shift away from

morbidity control and towards elimination, especially in areas of low endemicity, requires the implementation of a surveillance and response system geared towards elimination. A surveillance and response system is a well-designed public health scheme that systematically collects, analyzes, and interprets disease and health data, uses this data to identify risk areas and populations most impacted by the disease, provides public health personnel with appropriate disease interventions, and measures the efficiency and impact of these interventions (Zhou et al. 2013). The elimination of lymphatic filariasis in the People's Republic of China is an example of successful implementation of a surveillance and response system for elimination (Bergquist et al. 2015). To function properly, a surveillance and response system requires reliable data on spatiotemporal distribution, prevalence, incidence, and disease burden, with the end result of disease transmission interruption and eventual elimination of infections within an entire community (Tambo et al. 2014). To achieve this, surveillance and response systems must incorporate more highly sensitive diagnostic testing to detect disease in low-transmission communities, and pair this with high-resolution modeling to evaluate disease risk and transmission within a given area.

2.5. References

Amoah ID, Singh G, Stenström TA, Reddy P. 2017. Detection and quantification of soil-transmitted helminths in environmental samples: A review of current state-of-the-art and future perspectives. Acta Trop. 169:187–201. doi:10.1016/j.actatropica.2017.02.014.

Barda B, Zepherine H, Rinaldi L, Cringoli G, Burioni R, Clementi M, Albonico M. 2013. Mini-FLOTAC and Kato-Katz: helminth eggs watching on the shore of Lake Victoria. Parasit. Vectors 6:220. doi:10.1186/1756-3305-6-220.

Barda BD, Rinaldi L, Ianniello D, Zepherine H, Salvo F, Sadutshang T, Cringoli G, Clementi M, Albonico M. 2013. Mini-FLOTAC, an Innovative Direct Diagnostic Technique for Intestinal Parasitic Infections: Experience from the Field. PLoS Negl. Trop. Dis. 7:e2344. doi:10.1371/journal.pntd.0002344.

Bergquist R, Yang GJ, Knopp S, Utzinger J, Tanner M. 2015. Surveillance and response: Tools and approaches for the elimination stage of neglected tropical diseases. Acta Trop. 141:229–234. doi:10.1016/j.actatropica.2014.09.017.

Bethony J, Brooker S, Albonico M, Geiger SM, Loukas A, Diemert D, Hotez PJ. 2006. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. Lancet 367:1521–1532. doi:10.1016/S0140-6736(06)68653-4.

Blouin B, Casapía M, Joseph L, Kaufman JS, Larson C, Gyorkos TW. 2018. The effect of cumulative soil-transmitted helminth infections over time on child development: A 4-year longitudinal cohort study in preschool children using Bayesian methods to adjust for exposure misclassification. Int. J. Epidemiol. 47:1180–1194. doi:10.1093/ije/dyy142.

Bonne C. 1937. Invasion of the Submucosa of the Human Small Intestine by Ancylostoma Braziliense. Am. J. Trop. Med. Hyg. s1-17:587–594. doi:10.4269/ajtmh.1937.s1-17.587.

Brazilian Ministry of Health. 2012. Integrated Strategic Action Plan for the Elimination fo Leprosy, Filariasis, Schistosomiasis and Onchocerciasis as a Public Health Problem, Trachoma as a Cause of Blindness and Control of Geohelmithiases: Action Plan 2011-2015. Brasilia, Brazil.

Brelsford JB, Plieskatt JL, Yakovleva A, Jariwala A, Keegan BP, Peng J, Xia P, Li G, Campbell D, Periago MV, et al. 2017. Advances in neglected tropical disease vaccines: Developing relative potency and functional assays for the Na-GST-1/Alhydrogel hookworm vaccine. Bentwich Z, editor. PLoS Negl. Trop. Dis. 11:e0005385. doi:10.1371/journal.pntd.0005385.

Brooker S. 2010. Estimating the global distribution and disease burden of intestinal nematode infections: adding up the numbers--a review. Int. J. Parasitol. 40:1137–44. doi:10.1016/j.ijpara.2010.04.004.

Brooker S, Beasley M, Ndinaromtan M, Madjiouroum EM, Baboguel M, Djenguinabe E, Hay SI, Bundy DAP. 2002. Use of remote sensing and a geographical information system in a national helminth control programme in Chad.

Brooker S, Clements ACA, Bundy DAP. 2006. Global Epidemiology, Ecology and Control of Soil-Transmitted Helminth Infections. Adv. Parasitol. 62:221–261. doi:10.1016/S0065-308X(05)62007-6.

Brooker S, Hotez PJ, Bundy DAP. 2008. Hookworm-Related Anaemia among Pregnant Women: A Systematic Review. Raso G, editor. PLoS Negl. Trop. Dis. 2:e291. doi:10.1371/journal.pntd.0000291.

Carroll SM, Grove DI. 1986. Experimental infection of humans with Ancylostoma ceylanicum: Clinical, parasitological, haematological and immunological findings. Trop. Geogr. Med. 38:38–45.

Chaia G, Chaia AB, McAullife J, Katz N, Gasper D. 1968. Coprological diagnosis of schistosomiasis. II. Comparative study of quantitative methods. Rev. Inst. Med. Trop. Sao Paulo 10:349–353.

Chammartin F, Guimarães LH, Scholte RGC, Bavia ME. Spatio-temporal distribution of soil-transmitted helminth infections.

Chammartin F, Guimarães LH, Scholte RGC, Bavia ME, Utzinger J, Vounatsou P. 2014. Spatio-temporal distribution of soil-transmitted helminth infections in Brazil. Parasites and Vectors 7:440. doi:10.1186/1756-3305-7-440.

Crompton DW. 2000. The public health importance of hookworm disease. Parasitology 121 Suppl:S39-50. doi:10.1017/S0031182000006454.

Cunningham LJ, Odoom J, Pratt D, Boatemaa L, Asante-Ntim N, Attiku K, Banahene B, Osei-Atweneboana M, Verweij JJ, Molyneux D, et al. 2018. Expanding molecular diagnostics of helminthiasis: Piloting use of the GPLN platform for surveillance of soil transmitted helminthiasis and schistosomiasis in Ghana. Bottazzi ME, editor. PLoS Negl. Trop. Dis. 12:e0006129. doi:10.1371/journal.pntd.0006129.

Diemert DJ, Freire J, Valente V, Fraga CG, Talles F, Grahek S, Campbell D, Jariwala A, Periago MV, Enk M, et al. 2017. Safety and immunogenicity of the Na-GST-1 hookworm vaccine in Brazilian and American adults. McCarthy JS, editor. PLoS Negl. Trop. Dis. 11:e0005574. doi:10.1371/journal.pntd.0005574.

Dold C, Holland C V. 2011. Ascaris and ascariasis. Microbes Infect. 13:632–7. doi:10.1016/j.micinf.2010.09.012.

de Gier B, Nga TT, Winichagoon P, Dijkhuizen MA, Khan NC, van de Bor M, Ponce MC, Polman K, Wieringa FT. 2016. Species-Specific Associations Between Soil-Transmitted Helminths and Micronutrients in Vietnamese Schoolchildren. Am. J. Trop. Med. Hyg. 95:77–82. doi:10.4269/ajtmh.15-0533.

Hoffman M, Pons J, Janer J. 1934. The sedimentation-concentration method in schistosomiasis mansoni. PR. J. Public Health Trop. Med. 9:283–291.

Hotez P, Ottesen E, Fenwick A, Molyneux D. 2006. The neglected tropical diseases: The ancient afflictions of stigma and poverty and the prospects for their control and elimination. In: Advances in Experimental Medicine and Biology. Vol. 582. Springer US. p. 23–33.

Hotez PJ, Beaumier CM, Gillespie PM, Strych U, Hayward T, Bottazzi ME. 2016. Advancing a vaccine to prevent hookworm disease and anemia. Vaccine 34:3001–3005. doi:10.1016/j.vaccine.2016.03.078.

Hotez PJ, Diemert D, Bacon KM, Beaumier C, Bethony JM, Bottazzi ME, Brooker S, Couto AR, da Silva Freire M, Homma A, et al. 2013. The human hookworm vaccine. Vaccine 31:227–232. doi:10.1016/j.vaccine.2012.11.034.

Hotez PJ, Fenwick A, Savioli L, Molyneux DH. 2009. Rescuing the bottom billion through control of neglected tropical diseases. Lancet 373:1570–1575. doi:10.1016/s0140-6736(09)60233-6.

Jensen JR. 2007. Remote Sensing of the Environment: An Earth Resource Perspective. 2nd ed. Upper Saddle River, NJ: Pearson Prentice Hall.

Jourdan PM, Lamberton PHL, Fenwick A, Addiss DG. 2018. Soil-transmitted helminth infections. Lancet 391:252–265. doi:10.1016/S0140-6736(17)31930-X.

Kassebaum NJ, Jasrasaria R, Naghavi M, Wulf SK, Johns N, Lozano R, Regan M, Weatherall D, Chou DP, Eisele TP, et al. 2014. A systematic analysis of global anemia burden from 1990 to 2010. Blood 123:615–24. doi:10.1182/blood-2013-06-508325.

Kato K, Miura M. 1954. Comparative examinations. Japanese J. Parasitol. 3:35.

Katz N, Chaves A, Pellegrino J. 1972. A simple device for quantitative stool thick-smear technique in Schistosomiasis mansoni. Rev. Inst. Med. Trop. Sao Paulo 14:397–400. doi:10.1590/S0036-46651999000200001.

Knopp S, Glinz D, Rinaldi L, Mohammed KA, N'Goran EK, Stothard JR, Marti H, Cringoli G, Rollinson D, Utzinger J. 2009. FLOTAC: A promising technique for detecting helminth eggs in human faeces. Trans. R. Soc. Trop. Med. Hyg. 103:1190–1194. doi:10.1016/j.trstmh.2009.05.012.

Kongs A, Marks G, Verlé P, Van Der Stuyft P. 2001. The unreliability of the Kato-Katz technique limits its usefulness for evaluating S. mansoni infections. Trop. Med. Int. Heal. 6:163–169. doi:10.1046/j.1365-3156.2001.00687.x.

Levecke B, Behnke JM, Ajjampur SSR, Albonico M, Ame SM, Charlier J, Geiger SM, Hoa NTV, Ngassam RIK, Kotze AC, et al. 2011. A comparison of the sensitivity and fecal egg counts of the McMaster egg counting and Kato-Katz thick smear methods for soil-transmitted helminths. de Silva N, editor. PLoS Negl. Trop. Dis. 5:e1201. doi:10.1371/journal.pntd.0001201.

Lutz A. 1919. Schistosomum Mansoni and Schistosomatosis observed in Brazil. Mem. Inst. Oswaldo Cruz 11:121–155.

Manz KM, Clowes P, Kroidl I, Kowuor DO, Geldmacher C, Ntinginya NE, Maboko L, Hoelscher M, Saathoff E. 2017. Trichuris trichiura infection and its relation to environmental factors in Mbeya region, Tanzania: A cross-sectional, population-based study. Diemert DJ, editor. PLoS One 12:e0175137. doi:10.1371/journal.pone.0175137.

Martin LK, Beaver PC. 1968. Evaluation of Kato thick-smear technique for quantitative diagnosis of helminth infections.

Martins-Melo FR, Ramos AN, Alencar CH, Lima MS, Heukelbach J. 2017. Epidemiology of soil-transmitted helminthiases-related mortality in Brazil. Parasitology 144:669–679. doi:10.1017/S0031182016002341.

McCarthy JS, Cimino R, Williams SA, Mejia R, Llewellyn S, Grant JR, Papaiakovou M, Krolewiecki AJ, Traub RJ, Pilotte N. 2017. A novel, species-specific, real-time PCR assay for the detection of the emerging zoonotic parasite Ancylostoma ceylanicum in human stool. Bethony JM, editor. PLoS Negl. Trop. Dis. 11:e0005734. doi:10.1371/journal.pntd.0005734.

Mejia R, Vicuña Y, Broncano N, Sandoval C, Vaca M, Chico M, Cooper PJ, Nutman TB. 2013. A novel, multi-parallel, real-time polymerase chain reaction approach for eight gastrointestinal parasites provides improved diagnostic capabilities to resource-limited at-risk populations. Am. J. Trop. Med. Hyg. 88:1041–1047. doi:10.4269/ajtmh.12-0726.

Mudenda NB, Malone JB, Kearney MT, Mischler PD, Del P, Nieto M, McCarroll JC, Vounatsou P. 2012. Modelling the ecological niche of hookworm in brazil based on climate. Geospat. Health 6.

Murray CJL. 1994. Quantifying the burden of disease: The technical basis for disability-adjusted life years. Geneva.

Murray CJL, Ezzati M, Flaxman AD, Lim S, Lozano R, Michaud C, Naghavi M, Salomon JA, Shibuya K, Vos T, et al. 2012. GBD 2010: Design, definitions, and metrics.

Murray CJL, Ezzati M, Flaxman AD, Lim S, Lozano R, Michaud C, Naghavi M, Salomon JA, Shibuya K, Vos T, et al. 2012. Supplement to: Murray CJL, Ezzati M, Flaxman AD, et al. GBD 2010: design, definitions, and metrics.

Murray CJL, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, Ezzati M, Shibuya K, Salomon JA, Abdalla S, et al. 2012. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: A systematic analysis for the Global Burden of Disease Study 2010.

Nejsum P, Betson M, Bendall RP, Thamsborg SM, Stothard JR. 2012. Assessing the zoonotic potential of Ascaris suum and Trichuris suis: looking to the future from an analysis of the past. J. Helminthol. 86:148–155. doi:10.1017/S0022149X12000193.

O'Connell EM, Mitchell T, Papaiakovou M, Pilotte N, Lee D, Weinberg M, Sakulrak P, Tongsukh D, Oduro-Boateng G, Harrison S, et al. 2018. Ancylostoma ceylanicum hookworm in myanmar refugees, Thailand, 2012–2015. Emerg. Infect. Dis. 24:1472–1481. doi:10.3201/eid2408.180280.

O'LORCAIN P, HOLLAND CV. 2002. The public health importance of Ascaris lumbricoides. Parasitology 121:S51. doi:10.1017/s0031182000006442.

Pabalan N, Singian E, Tabangay L, Jarjanazi H, Boivin MJ, Ezeamama AE. 2018. Soil-transmitted helminth infection, loss of education and cognitive impairment in school-aged children: A systematic review and meta-analysis. Budke CM, editor. PLoS Negl. Trop. Dis. 12:e0005523. doi:10.1371/journal.pntd.0005523.

Phillips SJ, Anderson RP, Dudík M, Schapire RE, Blair ME. 2017. Opening the black box: an open-source release of Maxent. Ecography (Cop.). 40:887–893. doi:10.1111/ecog.03049.

Phillips SJ, Anderson RP, Schapire RE. 2006. Maximum entropy modeling of species geographic distributions. Ecol. Modell. 190:231–259. doi:10.1016/j.ecolmodel.2005.03.026.

Phillips SJ, Dudík M, Schapire RE, Avenue P, Park F, Dudík M, Schapire RE. 2004. A maximum entropy approach to species distribution modeling. Twenty-first Int. Conf. Mach. Learn. - ICML '04:83. doi:10.1145/1015330.1015412.

Pullan RL, Smith JL, Jasrasaria R, Brooker SJ. 2014. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. Parasit. Vectors 7:37. doi:10.1186/1756-3305-7-37.

de Silva NR, Brooker S, Hotez PJ, Montresor A, Engels D, Savioli L. 2003. Soil-transmitted helminth infections: updating the global picture. Trends Parasitol. 19:547–551. doi:10.1016/j.pt.2003.10.002.

de Silva NR, Chan MS, Bundy D a. 1997. Morbidity and mortality due to ascariasis: re-estimation and sensitivity analysis of global numbers at risk. Trop. Med. Int. Health 2:519–28. doi:10.1046/j.1365-3156.1997.d01-320.x.

Smout FA, Skerratt LF, Butler JRA, Johnson CN, Congdon BC, Thompson RCA. 2017. The hookworm Ancylostoma ceylanicum: An emerging public health risk in Australian tropical rainforests and Indigenous communities. One Heal. 3:66–69. doi:10.1016/j.onehlt.2017.04.002.

Soares Magalhães RJ, Salamat MS, Leonardo L, Gray DJ, Carabin H, Halton K, McManus DP, Williams GM, Rivera P, Saniel O, et al. 2015. Mapping the Risk of Soil-Transmitted Helminthic Infections in the Philippines. PLoS Negl. Trop. Dis. 9:e0003915. doi:10.1371/journal.pntd.0003915.

Speich B, Knopp S, Mohammed KA, Khamis IS, Rinaldi L, Cringoli G, Rollinson D, Utzinger J. 2010. Comparative cost assessment of the Kato-Katz and FLOTAC techniques for soil-transmitted helminth diagnosis in epidemiological surveys. Parasit. Vectors 3:71. doi:10.1186/1756-3305-3-71.

Stiles CW. 1902a. A new species of hookworm (Uncinaria americana) parasitic in man.

Stiles CW. 1902b. Hookworm disease in the South-frequency of infection by the parasite (uncianaria americana) in rural districts. Public Health Rep. 17:181–182. doi:10.1525/tph.2001.23.2.29.

Tambo E, Ai L, Zhou X, Chen J-H, Hu W, Bergquist R, Guo J-G, Utzinger J, Tanner M, Zhou X-N. 2014. Surveillance-response systems: the key to elimination of tropical diseases. Infect. Dis. poverty 3:17. doi:10.1186/2049-9957-3-17.

Tang YT, Gao X, Rosa B a, Abubucker S, Hallsworth-Pepin K, Martin J, Tyagi R, Heizer E, Zhang X,

Bhonagiri-Palsikar V, et al. 2014. Genome of the human hookworm Necator americanus. Nat. Genet. 46:261–9. doi:10.1038/ng.2875.

The Rockefeller Foundation-International Health Board. 1922. Bibliography of Hookworm Disease. New York City.

Tomlinson RF. 1969. A Geographic Information System for Regional Planning.

Turner HC, Truscott JE, Bettis AA, Shuford K V, Dunn JC, Hollingsworth TD, Brooker SJ, Anderson RM. 2015. An economic evaluation of expanding hookworm control strategies to target the whole community. Parasit. Vectors 8:570. doi:10.1186/s13071-015-1187-5.

Warren DL, Seifert SN. 2011. Ecological niche modeling in Maxent: The importance of model complexity and the performance of model selection criteria. Ecol. Appl. 21:335–342. doi:10.1890/10-1171.1.

WHO. 1991. Basic laboratory methods in medical parasitology. WHO.

WHO. 1994. Bench aids for the diagnosis of intestinal parasites. Geneva: World Health Organization.

WHO. 2002. Prevention and control of schistosomiasis and soil-transmitted helminthiasis : report of a WHO expert committee. Geneva: World Health Organization.

WHO. 2004. Action against worms N°4: How to set up a deworming programme. Allen H, editor. Geneva: World Health Organization.

WHO. 2011. Helminth control in school-age children. A guide for managers of control programmes. 2nd ed. Geneva: World Health Organization.

WHO. 2012. Soil-transmitted helminthiases : eliminating as public health problem soil-transmitted helminthiases in children : progress report 2001-2010 and strategic plan 2011-2020. Geneva: World Health Organization.

Zhou XN, Bergquist R, Tanner M. 2013. Elimination of tropical disease through surveillance and response. Infect. Dis. Poverty 2:1–5. doi:10.1186/2049-9957-2-1.

Chapter 3. Comparison of Diagnostic Techniques for Quantifying Intensity of Soil-Transmitted Helminth Infections in Feira de Santana, Brazil

3.1. Introduction

STH infections, primarily caused by the roundworm *A. lumbricoides*, the hookworm species *N. americanus* and *A. duodenale*, and the whipworm *T. trichiura,* affect over 1.5 billion people, with billions more at risk, especially in warm, tropical climates (de Silva et al. 2003; Brooker 2010). STH infections are transmitted via either a direct fecal-oral (*A. lumbricoides*, *T. trichiura*) or a fecal-cutaneous (*N. americanus*, *A. duodenale*) route, and are closely linked with poverty, poor hygiene, and poor sanitation. While often asymptomatic, symptomatic STH infections present with intestinal pain, malnutrition, and impaired cognitive and physical development (Bethony et al. 2006). During the migratory phase through the lungs, larval *A. lumbricoides* may cause pulmonary eosinophilic pneumonitis known as Loeffler syndrome, which can lead to severe morbidity and even death (Akuthota and Weller 2012).

The country of Brazil has no national STH control effort, leaving states and municipalities to decide whether to fund and enact programs. Due to the relatively low estimated prevalence across the majority of Brazil (<20%) (Chammartin et al. 2014), most areas rely on passive STH surveillance and incidental case-findings from local health clinics or the national Brazilian Schistosomiasis Control Program (Brazilian Ministry of Health 2012; Martins-Melo et al. 2017). The reliance on passive surveillance leads to the likely underestimation of the true STH infection prevalence since schistosome ranges do not always overlap with STHs ranges (Brazilian Ministry of Health 2012; Couto et al. 2014). Diagnostic testing for STH infection in Brazil currently relies mainly on the WHO recommended Kato-Katz thick smear method (WHO 1998; WHO 2011). The Kato-Katz thick smear was first described by Kato and Miura (1954), and then improved upon by

others, including Martin and Beaver (1968) and Chaia et al. (1968). The Kato-Katz technique has been advocated for years by the WHO and is the most widely-used method to determine STH prevalence, as it requires little equipment, is easy learn, and requires only a small amount of fecal material (WHO 2004). While used often, this method has caveats: a simultaneous assessment of all STH species is difficult due to the differential time intervals (clearing time) it takes for each type of STH egg to appear, the rapid clearing of hookworm eggs, if not examined within 30-45 minutes leads to false-negative results, and overall inconsistent egg detection and under-quantification (Booth et al. 2003; Knopp et al. 2008; Levecke et al. 2011). These issues are more likely if the Kato-Katz is not performed over consecutive days using consecutive fecal samples from the same patient (Assefa et al. 2014; Liu et al. 2017).

In the city of Feira de Santana, Brazil, located in the northeast state of Bahia, the MOH evaluates fecal samples uses the Kato-Katz technique. They evaluate only a single Kato-Katz slide from a patient's one collected stool sample. This increases the likelihood that true positives will be undetected. Previous research in Feira de Santana has estimated the STH infection prevalence to be <20% (Almeida et al. 2012), presenting a real opportunity to eliminate STH infections and interrupt transmission if these estimates are correct. Conversely, if the <20% prevalence estimate is incorrect, more sensitive diagnostics can elucidate the true disease burden. To achieve this goal, an STH surveillance and response system geared towards elimination is needed to replace the current passive surveillance morbidity control program. We propose that an effective surveillance and response elimination system requires the use of higher-accuracy diagnostics than the Kato-Katz thick smear (Bergquist et al. 2015), the evaluation of entire households for

STH infections, and the ability to differentiate STH prevalence between different city communities.

This study evaluated three representative communities in Feira de Santana for STH infection using the MOH Kato-Katz method, as well as with two more recently developed diagnostic tests, the mini-FLOTAC method (B.D. Barda et al. 2013) and a multi-parallel quantitative polymerase chain reaction (qPCR) method (Mejia et al. 2013). This study set out to test the hypotheses: that STH prevalence varies depending on the type of community (urban, peri-urban, rural); that adults within households serve as overlooked reservoirs of STH infection; and that both the qPCR and mini-FLOTAC diagnostic methods provide increased STH infection detection rates compared to the MOH Kato-Katz method. This research provides the first step towards enacting an effective STH elimination surveillance and response system in Feira de Santana, Brazil.

3.2. Materials and Methods

The mini-FLOTAC method has demonstrated significantly higher STH egg sensitivity compared to the direct fecal smear and formol-ether concentration diagnostic methods, and equivalent to the Kato-Katz method (B.D. Barda et al. 2013; B. Barda et al. 2013; Barda et al. 2014). It provides the added benefits of a quicker examination time and the ability to evaluate preserved samples compared to the Kato-Katz. The qPCR method allows for detection and quantification of multiple types of parasites and is optimized to allow for inexpensive analysis of each sample. Previous work has demonstrated that qPCR has significantly higher sensitivity for *A. duodenale* and *T. trichiura* eggs than the saline wet mount method (Mejia et al. 2013) and

significantly higher hookworm egg sensitivity than either the Telemann concentration or McMaster diagnostic methods (Cimino et al. 2015).

3.2.1. Ethics Statement

Research protocols were approved by the Louisiana State University Institution Review Board Include IRB #3633 (Appendix A.) and the National Research Ethics Commission of Brazil and CONEP #54801816.0.1001.0053 (Appendix B.). Field and laboratory practices were described to the Ministry of Health team in Feira de Santana that aided in collection and analysis. All study participants were unpaid and voluntarily agreed to participate with the stipulation of withdrawal at any time. Oral explanations on the details of the study were given to adults in the household with the knowledge that positive cases would be treated by the Ministry of Health free of charge. Written consent was obtained from all participants, as well as child assent forms and guardian consent forms for children enrolled in the study (Appendices H., J., L.).

3.2.2. Study Area and Representative Communities

The study area consisted of three sites within the city of Feira de Santana, Brazil, which is in northeast Brazil (Figure 3.1.A.). Feira de Santana is the second largest city in the state of Bahia and straddles the biomes of caatinga and Bahian coastal forest. Caatinga is characterized by arid, hot season temperatures most of the year and pale, withered scrubland (Castelletti et al. 2004; Benício et al. 2015). The Bahian coastal forest is part of the larger Brazilian Atlantic forest and comprised of moist and semi-deciduous forests, with a hot, humid climate and evenly distributed rainfall throughout the year (Morellato and Haddad 2006). The study sites consisted of an urban community, Queimadinha, a peri-urban community, GDGN, and a rural community, Maria

Quiteria (Figure 3.1.B.). These communities were chosen to allow evaluation and comparison of different types of communities found within Feira de Santana.

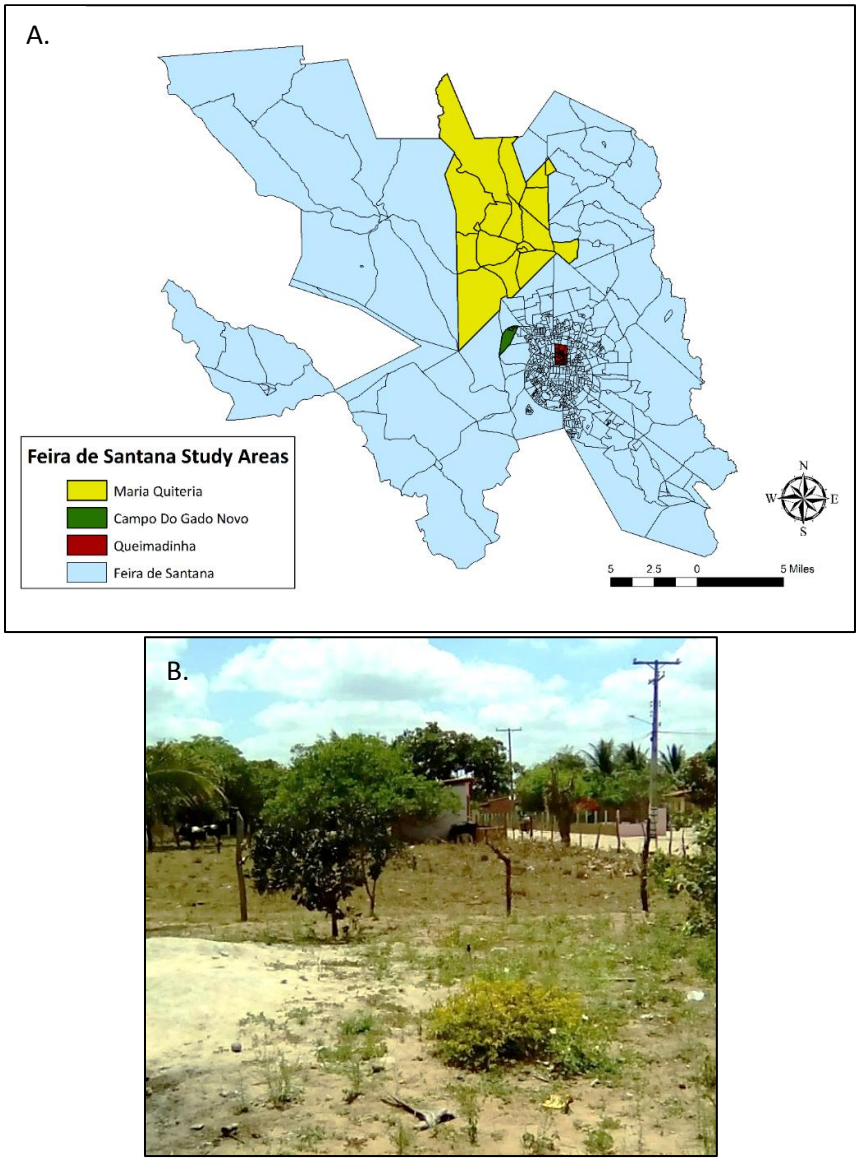

Figure 3.1. (A.) The city of Feira de Santana, Brazil and the three representative communities of Maria Quiteria, Campo Do Gado Novo (CDGN), and Queimadinha sampled in this study. (B.) An example of Feira de Santana ecology from the community of Maria Quiteria.

3.2.3. Field Study Design and Sampling

Household sampling was conducted across all study sites in conjunction with the Feira de Santana Ministry of Health personnel. Sampling methodology followed established Feira de

Santana Ministry of Health practice. This consisted of active sampling of the community during the mornings until 12:00 pm, with personnel sampling participants that were present at the domicile during that time. If the domicile was vacant, the next available domicile would be sampled. At the start of each day, a different, non-random starting point within the community was chosen by the MOH to approximate as close to a spatially-distributed sample cohort as possible. Sampling was conducted within a study site until the statistically predetermined number of households and people were reached, based on the total population of each area and average person per household (Table 3.1).

Maria Quiteria encompasses a much larger area of land than CDGN or Queimadinha, and sampling originated at the local school. Sampling incorporated the school children and their mothers. All people sampled were then traced back to the respective sub-community, and then surrounding households within the sub-community were sampled using the methodology described above. This provided a spatially distributed collection of samples across the large, rural area.

The total number of houses sampled across all 3 communities was 97 and was comprised of 279 total participants (Table 3.1.). In both CDGN and Maria Quiteria, fecal samples collected from households (20 and 54, respectively) and people (69 and 134, respectively), exceeded the calculated sampling total necessary for a powered study (Table 3.1.). Calculated totals were determined using a conservative approach of 50% expected prevalence, with a 95% confidence interval, and 5% precision. Due to social unrest within the urban Queimadinha community, the MOH terminated sampling early and only 23 total households and 76 people were sampled.

Table 3.1. The total population and people per house for Maria Quiteria, Campo Do Gado Novo, and Queimadinha from 2010 census and the number of houses and people, both calculated and obtained, sampled in each community. Sample size was estimated based on 50% expected prevalence, 95% confidence interval, 5% precision.

Community	Total Population	People/House	Houses		People	
			Calculated	Obtained	Calculated	Obtained
Maria Quiteria	13853	3.73	33	54	124	134
CDGN	3832	2.32	15	20	34	69
Queimadinha	17684	2.49	63	23	158	76
Total	35369		111	97	316	279

Samples were obtained from all household members regardless of age, since the purpose was to collect a representative sample of the entire household, pre-SAC and SAC. Participants provided answers to Ministry personnel for both a household questionnaire and an individual questionnaire applied to every willing participant within the household (Appendices D., F.). Sample cups and applicator sticks were left for fecal sample collection and then picked up the following day by the field team. Samples were taken to the lab at Universidade Estadual do Feira de Santana (UEFS) where they were stored in a 4° C refrigerator until evaluation.

Maps of the sampling distribution for Queimadinha (Figure 3.2.), CDGN (Figure 3.3.), and Maria Quiteria (Figure 3.4.) were created in ArcMap 10.6 (ESRI) using the Kernel Density tool to illustrate the final sampling distribution. The output values were densities and the method was planar for all maps. The cell size was set to 0.5 m^2 for Queimadinha and CDGN, and the search radius was set to 207 m^2 for Queimadinha and 211 m^2 for CDGN based on the average household-habitat area calculated. For the larger area of Maria Quiteria, the cell size was set to 2 m^2 and the search was set 1500 m^2 to account for the distance between different farm sub-communities sampled.

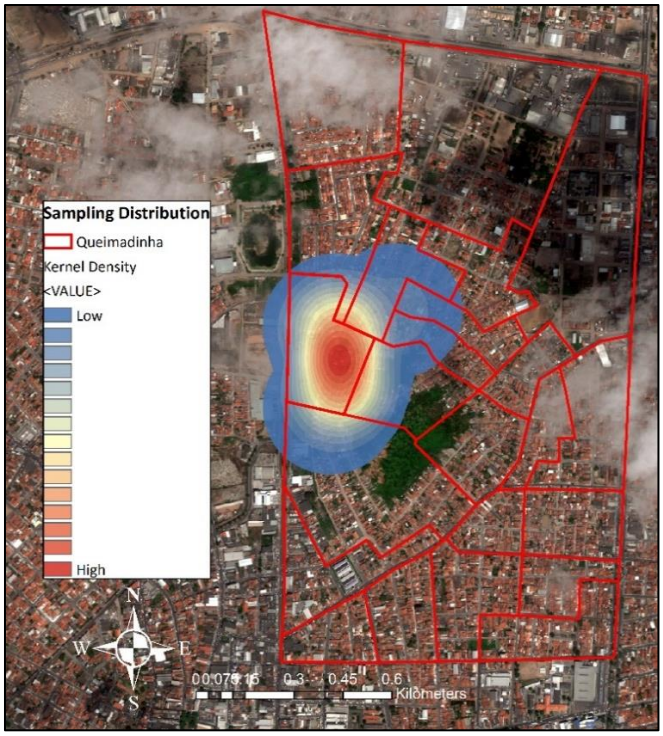

Figure 3.2. The sampling distribution map for the community of Queimadinha created using kernel density estimation. Copyright 2016 DigitalGlobe NextView License

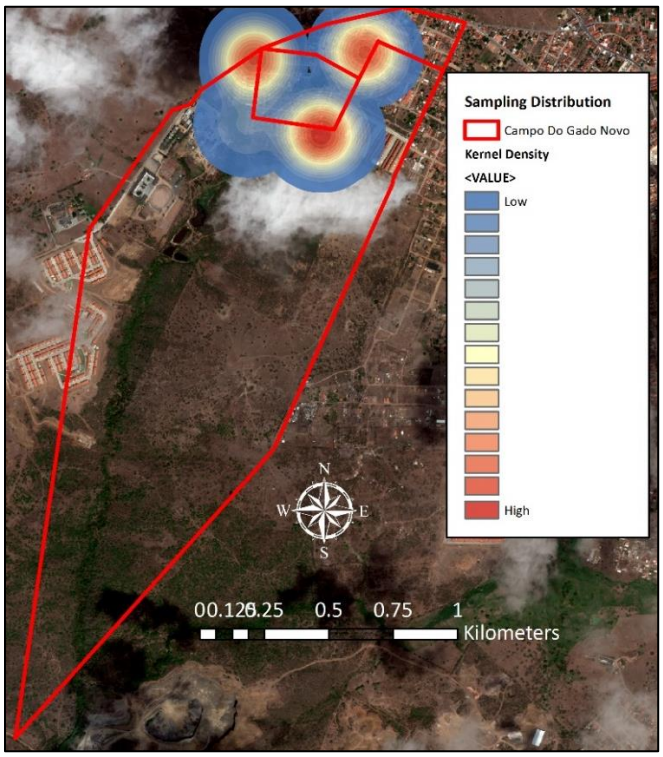

Figure 3.3. The sampling distribution map for the community of Campo Do Gado Novo created using kernel density estimation. Copyright 2016 DigitalGlobe NextView License

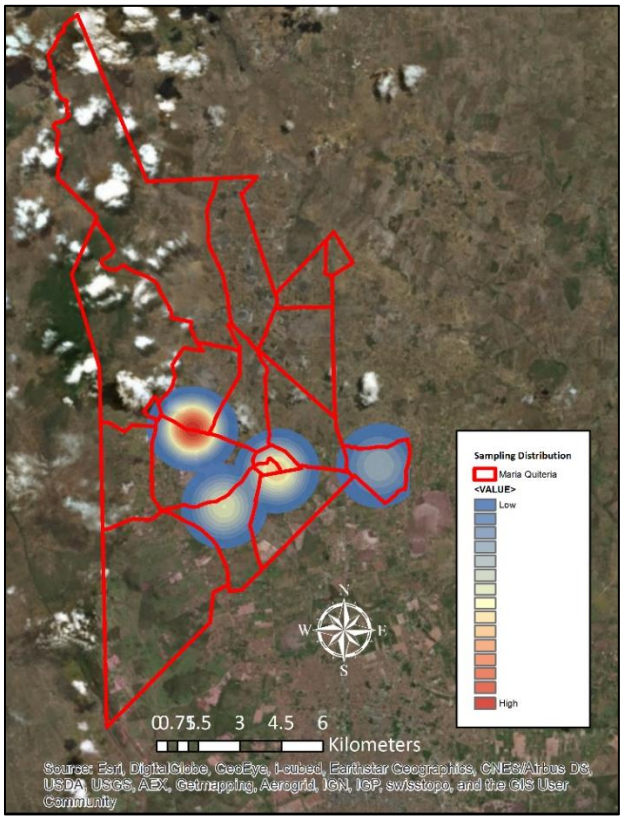

Figure 3.4. The sampling distribution map for the community of Maria Quiteria created using kernel density estimation. Copyright 2016 DigitalGlobe NextView License

3.2.4. Diagnostic Techniques

Samples collected from the field were analyzed using the classical WHO recommended Kato-Katz thick smear (Kato and Miura 1954; Katz et al. 1972; WHO 1991), the mini-FLOTAC diagnostic technique (B.D. Barda et al. 2013; B. Barda et al. 2013; Barda et al. 2014), and a quantitative multi-parallel PCR (qPCR) test (Mejia et al. 2013; Cimino et al. 2015; Weatherhead et al. 2017).

3.2.4.1. Kato-Katz Thick Smear

The WHO recommends the Kato-Katz thick smear due to its simple, straightforward approach, which requires minimal equipment and training to carry out (Figure 3.5.) (WHO 2004;

Speich et al. 2010). The technique was described by Ash and Orihel in the Bench Aids for the diagnosis of intestinal parasites (WHO 1994). In this study, Kato-Katz smears were prepared and evaluated by MOH personnel, with preparation consisting of a small sieve that filters the fecal sample, a 41.7 mg special template that regularized the amount of fecal material used, recommended by the WHO (1994) to ensure repeatability and comparability, a microscope slide, cellophane, and glycerol-methylene blue or glycerol-malachite green. The sieve filtered the feces which was then smeared through the template hole onto a microscope slide. The slide was covered with cellophane soaked in glycerol-malachite green or glycerol-methylene blue solution and then pressed face down to evenly distribute the feces. Slides were left to dry at an ambient temperature of ~24° C to clear fecal material and were evaluated by the MOH the following week. Total number of eggs counted was then multiplied by 24, the multiplication factor for a 41.7 mg template, to obtain the eggs per gram (EPG) count of each STH.

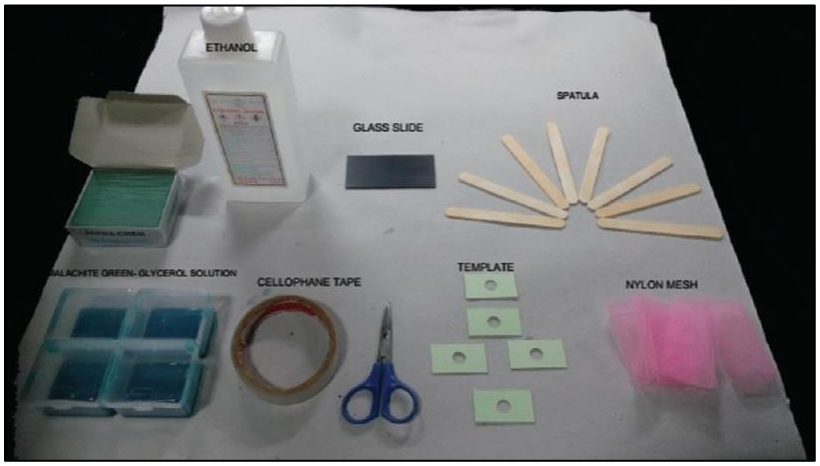

Figure 3.5. An example of the Kato-Katz thick smear kit (Courtesy of Tropical Parasitology http://www.tropicalparasitology.org/viewimage.asp?img=TropParasitol_2017_7_2_111_215506_f2.jpg)

3.2.4.2. The mini-FLOTAC Flotation Method

The mini-FLOTAC method was created by Barda. et. al. (2013), as a simple diagnostic method for STH infections to address the challenge of implementing a high-sensitivity and

affordable test in resource-limited areas of the world. The mini-FLOTAC method is a homogenization and passive flotation technique that allows for quick, inexpensive examination of samples with minimal equipment (Figure 3.6.). It is a simplified version of the FLOTAC technique (Cringoli 2006; Utzinger et al. 2008; Knopp et al. 2009), that eliminates the added FLOTAC complexity and the need for a specific centrifugation device. The mini-FLOTAC requires neither a centrifugation step nor expensive equipment, can be performed on either fixed or fresh stool samples, and can be quickly evaluated within 15-20 minutes (B.D. Barda et al. 2013). Evaluation of samples via mini-FLOTAC was conducted by a trained researcher in the UEFS Parasitology laboratory using both the FS2 solution (NaCl, specific gravity 1.20) and FS7 solution ($ZnSO_4$, 1.35 specific gravity) as flotation media. These solutions were selected due to their usefulness in diagnosing STH (FS2) and *S. mansoni* and protozoa (FS7), as demonstrated previously by Cringoli (2006). For this study, the NaCl and $ZnSO_4$ solutions were compared to each other to assess both STH detection rates and the EPG counts obtained by each solution. The mini-FLOTAC kit contains a fill-FLOTAC, which is a disposable homogenizing container that also weighs, filters, and fills the mini-FLOTAC, and a mini-FLOTAC itself, a small disposable disc which consists of two 1-ml flotation chambers where the passive flotation of parasite eggs and oocysts occurs (Figure 3.3.). The mini-FLOTAC can be mounted on a microscope using a plastic microscope mounting apparatus and allows for microscopic examination at up to 400x magnification. For this study, which examined fresh human feces, 2 grams of stool was homogenized with 38 ml of flotation media in the fill-FLOTAC device. The homogenized fecal suspension was then used to fill both chambers of the mini-FLOTAC apparatus. After 10 minutes, both viewing chambers of the mini-FLOTAC were examined using a compound microscope to ascertain STH egg counts. Egg

counts were then multiplied by 10 to obtain EPG counts (B.D. Barda et al. 2013; Veterinary Parasitology and Parasitic Diseases). This was repeated using both solution media.

Figure 3.6. The fill-FLOTAC homogenizer and mini-FLOTAC reading disc apparatus [Courtesy of (B.D. Barda et al. 2013); doi:10.1371/journal.pntd.0002344.g001]

3.2.4.3. Multi-Parallel, Real-Time Quantitative PCR

The qPCR diagnostic method, developed by Mejia et al. (2013), allows for detection and quantification of multiple types of gastrointestinal parasites and was optimized for the inexpensive analysis of each sample.

To run the qPCR test, a 50 mg subsample of participants fecal samples were collected and frozen at -20° C. Samples stored at UEFS were transferred to the Universidade Federal da Bahia (UFBA), in Salvador, Brazil, for DNA extraction using the MP Biomedicals™ FastDNA™spin kit for soil (Figure 3.7.)(MP Biomedicals LLC, Santa Ana, CA) with a modified extraction protocol to minimize the amount of sample and reagents used, and omit the additional *T. trichiura* extraction step outlined by Mejia et al. (2013). The modified protocol added 978 µl sodium phosphate

buffer, 122 µl MT buffer, and 50 mg of stool to the lysing matrix E tube, then homogenized the tube using the Disruptor Genie (Scientific Industries Inc, Bohemia, NY) for 5 minutes at 3000 revolutions per minute (rpm). Following homogenization, samples were centrifuged at 14,000 g for 10 minutes, then 250 µl PPS was added to a new 2 ml tube. Supernatant from the centrifuged lysing matrix E tube was pipetted into this new 2 ml tube and inverted 10 times by hand. This new tube was centrifuged at 14,000 g for 10 minutes, then 2 µl of internal control (PBr322, 10^4) were added to the tube. Two new tubes were used and 500 µl of Binding matrix were added to them. The supernatant from the centrifuged tube was added to each binding matrix tube evenly and inverted for 2 minutes. The supernatant was removed from each tube, leaving approximately 1 µl of volume behind. The binding matrices were removed and added to a spin filter with tube catch and centrifuged for 2 minutes at 14,000 g. Catch was emptied and 500 µl of prepared SEWS-M was added to matrix, mixed with gentle pipetting. This was centrifuged for 2 minutes at 14,000 g, the catch tube fluid was discarded, and then the tube was centrifuged at 14,000 g for 2 minutes again. The current catch tube was replaced with a different catch tube and filter was left to air dry at room temperature (~24°C) for 5 minutes. 100 µl of DES was added and gently mixed with matrix, then centrifuged at 14,000 g for 2 minutes. Extracted samples were then stored at -20°C and then transported to the Baylor College of Medicine, Houston, Texas, for qPCR analysis using an ABI ViiA 7 Real-Time PCR System (Life Technologies Corporation, Grand Island, NY).

For detection and quantification of the extracted samples, species-specific primers and FAM-labeled minor groove binder probes were used for each STH parasite. The forward primer, reverse primer, and probe sequences for *A. lumbricoides* were TGCACATAAGTACTATTTGCGCGTAT (forward primer 5'->3'), CCGCCGACTGCTATTACATCA

(reverse primer 5'->3'), GAGCCACATAGTAAATT (FAM probe 5'->3') and targeted the ribosomal DNA internal transcribed spacer 1 (ITS-1) region (GenBank AB571301.1) of the *A. lumbricoides* genome (Table 3.2.) (Arizono et al. 2010).

Figure 3.7. The MP Biomedicals™ FastDNA™spin kit used to extract soil-transmitted helminth egg DNA from stool samples collected in Feira de Santana, Brazil. (Photo courtesy of Thermo Fisher Scientific https://assets.fishersci.com/TFS-Assets/CCG/product-images/F303622~p.eps-650.jpg)

For *T. trichiura,* the ribosomal DNA ITS-1 region (GenBank FM991956.1) (Cutillas et al. 2009) was also targeted using the forward primer TCCGAACGGCGGATCA (forward primer 5'->3'), the reverse primer CTCGAGTGTCACGTCGTCCTT (reverse primer 5'->3'), and the probe sequence TTGGCTCGTAGGTCGTT (FAM probe 5'->3') (Figure 3.2.). For both hookworm species, the ribosomal DNA ITS-2 region was targeted. For the *A. duodenale* ITS-2 region (GenBank ITS-2 EU344797.1) (Basuni et al. 2011), the forward primer, reverse primer, and FAM probe sequences were GAATGACAGCAAACTCGTTGTTG (forward primer 5'->3'), ATACTAGCCACTGCCGAAACGT (reverse primer 5'->3'), and ATCGTTTACCGACTTTAG (FAM probe 5'->3') (Figure 3.2.). For the *N. americanus* ITS-2 region (GenBank ITS-2 AJ001599.1) (Basuni et al. 2011), the forward primer, reverse primer, and FAM probe sequences were CTGTTTGTCGAACGGTACTTGC (forward primer

5’->3’), ATAACAGCGTGCACATGTTGC (reverse primer 5’->3’), CTGTACTACGCATTGTATAC (FAM probe 5’->3’) (Figure 3.2.). All samples were run in duplicate using 96-well MicroAmp optical plates (Life Technologies Corporation, Grand Island, NY) and the ABI ViiA7 real-time PCR system (Life Technologies Corporation, Grand Island, NY) with default parameters and 40 cycles. Samples were considered negative if cycle threshold ($C_{T)}$ values were >38.This C_T was determined by testing the detectable limits of each assay using serial dilutions in which the appropriate parasite plasmid was included (Mejia et al. 2013). Parasite quantification was previously tested using plasmids containing the target sequences, which were purified and then quantified using an ND-1-000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE) (Mejia et al. 2013). These were diluted to fixed concentrations and serial dilutions were made to create a standard curve for comparison to unknown samples. Assays were validated previously by using genomic DNA spiked into known negative stool samples or known positive stool samples (Mejia et al. 2013). Quantification of infection was conducted measuring the fg/µl of parasite-specific DNA.

Table 3.2. The forward, reverse primer sequences, the FAM-labelled probe sequences, and the target gene regions used by the real-time quantitative PCR diagnostic method (Mejia et al. 2013) to detect and quantify *Ascaris lumbricoides*, *Necator americanus*, *Ancylostoma duodenale*, and *Trichuris trichiura* eggs.

Parasite	Primers and FAM-labelled Probe Sequences	Target Gene Region
A. lumbricoides	Forward 5’->3’: TGCACATAAGTACTATTTGCGCGTAT Reverse 5’->3’: CCGCCGACTGCTATTACATCA Probe 5’->3’: GAGCCACATAGTAAATT	rDNA ITS-1 region (GenBank AB571301.1)
N. americanus	Forward 5’->3’: CTGTTTGTCGAACGGTACTTGC Reverse 5’->3’: ATAACAGCGTGCACATGTTGC Probe 5’->3’: CTGTACTACGCATTGTATAC	rDNA ITS-2 region (GenBank ITS-2 AJ001599.1)
A. duodenale	Forward 5’->3’: GAATGACAGCAAACTCGTTGTTG Reverse 5’->3’: ATACTAGCCACTGCCGAAACGT Probe 5’->3’: ATCGTTTACCGACTTTAG	rDNA ITS-2 region GenBank ITS-2 EU344797.1)
T. trichiura	Forward 5’->3’: TCCGAACGGCGGATCA Reverse 5’->3’: CTCGAGTGTCACGTCGTCCTT Probe 5’->3’: TTGGCTCGTAGGTCGTT	rDNA ITS-1 region (GenBank FM991956.1)

3.2.5. Detection Rates of Diagnostic Tests

Detection rates were analyzed using the Generalized Linear Mixed Models (GLIMMIX) procedure in SAS 9.4 (SAS Institute Inc., Cary, NC) software. The detection rate is the ability of a diagnostic test to correctly identify positive infections. Comparison of parasite detection rates with GLIMMIX were assessed using the Least Squared Means, a binomial response distribution, a residual pseudo-likelihood estimation technique, and applying the Tukey-Kramer post-hoc test, which allows for pairwise analysis of means to calculate the minimum significant difference between pairs of means (McDonald 2014).

3.3. Results

3.3.1. Participant Demographics

A total of 279 people from 97 households were sampled across the 3 communities. Socioeconomic questionnaires were obtained from 250 of the 279 study participants. There were 114 male participants (45.6%) and 136 female participants (54.4%) with a mean participant age of 31.02 years and a range of <1-87 (Table 3.3.). Participants were divided into 15-year range groups except for the >60 range group.

Table 3.3. The distribution of participants in each age range across the communities of Queimadinha, Campo Do Gado Novo, and Maria Quiteria

Age Ranges	Number of Participants	Percentage
0-15	63	25.40
16-30	66	26.61
30-45	63	25.40
45-60	31	12.50
>60	25	10.08

3.3.2. Soil-Transmitted Helminth Infections Prevalence

Samples were considered positive if infection was detected by one or more of the diagnostic tests. Hookworm had the highest overall prevalence with 31 positive infections

(11.11%) (Table 3.4.), followed by 7 *A. lumbricoides* infections (2.51%), and 5 *T. trichiura* infections (1.79%) (Table 3.4.). The mean age of the infected participants was: 29.03 years for the 29 STH positive participants, 29.0 years for the 24 hookworm positive participants, 22.29 years for the 7 *A. lumbricoides* positive participants, and 19.2 years for the 5 *T. trichiura* positive participants (Table 3.5.).

Table 3.4. Number positive, total people sampled, and prevalence of each soil-transmitted helminth and across all 3 study communities. (Positive by ≥1 of the diagnostic tests)

Parasite	Positive	Total	Prevalence
Ascaris lumbricoides	7	279	2.51
Hookworm	31	279	11.11
Trichuris trichiura	5	279	1.79

Table 3.5. Number of soil-transmitted helminth, hookworm, *Ascaris lumbricoides*, and *Trichuris trichiura* infections and the mean age of infected participants.

Parasite Infection	Positive Participants	Mean Age
Any STH	29	29.03
Hookworm	24	29.0
A. lumbricoides	7	22.29
T. trichiura	5	19.20

3.3.3. qPCR Parasite Detection

A total of 98 samples were analyzed by the qPCR method, which enabled differentiation between the hookworm species. All 12 positive hookworm samples harbored *N. americanus* infections (12.24%), 4 samples were *A. lumbricoides* positive (4.08%), and 2 samples were *T. trichiura* positive 2.04% (Table 3.6.). The qPCR method enabled identification of additional gastrointestinal species. In addition to STH infections qPCR provides the ability to detect *Strongyloides stercoralis, Cryptosporidium parvum/hominis, Entamoeba histolytica, Giardia lamblia*, and *Blastocystis hominis* infections. In this study, *B. hominis*, *S. stercoralis*, and *G. lamblia* infections were detected along with the STH infections.

Table 3.6. The people positive, total people sampled, and percent positive for each soil-transmitted helminth by the real-time quantitative PCR test.

	Positive	Total	Percentage Positive
Necator americanus	12	98	12.24
Ancylostoma duodenale	0	98	0.00
Ascaris lumbricoides	4	98	4.08
Trichuris trichiura	2	98	2.04

3.3.4. A Comparison of Diagnostic Test Detection Rates

STH detection rates were compared between the Kato-Katz thick smear, the mini-FLOTAC flotation using both solutions, and the qPCR test. Each method was tested on the same fecal sample to allow direct comparisons across all test methods employed. The number of direct comparisons differed between the pairs of tests because it was not possible with the time and manpower available to evaluate all samples using each diagnostic method. There were 79 direct comparisons between Kato-Katz and qPCR, 36 comparisons between Kato-Katz and the mini-FLOTAC using both flotation solutions, 28 comparisons between qPCR and the mini-FLOTAC using both flotation solutions, and 47 comparisons between the mini-FLOTAC NaCl and $ZnSO_4$ solutions.

For the detection rate comparison between Kato-Katz and qPCR, no significant difference was found for hookworm, *A. lumbricoides*, or *T. trichiura* infections (Table 3.7.). Kato-Katz detected in 3/79 (3.8%) hookworm infections, 2/79 (2.53%) *A. lumbricoides* infections, and 1/79 (1.27%) *T. trichiura* infections (Table 3.7.). The qPCR test detected 9/78 (11.39%) hookworm infections, 4/79 (5.06%) *A. lumbricoides* infections, and 2/79 (2.53%) *T. trichiura* infections (Table 3.7.).

Table 3.7. The detection results for hookworm, *Ascaris lumbricoide*s, and *Trichuris trichiura* infections using the Kato-Katz thick smear and the quantitative PCR test.

Parasite	Test	Positive	Negative	Total	Percent Positive
Hookworm	Kato-Katz	3	76	79	3.8
	qPCR	9	70	79	11.39
Ascaris lumbricoides	Kato-Katz	2	77	79	2.53
	qPCR	4	75	79	5.06
Trichuris trichiura	Kato-Katz	1	78	79	1.27
	qPCR	2	77	79	2.53

For the detection rate comparison between Kato-Katz and the mini-FLOTAC using both $ZnSO_4$ and NaCl solutions, no significant difference was found for hookworm infections (Table 3.8.). The group of comparative samples examined via mini-FLOTAC and Kato-Katz were negative for both *A. lumbricoides* or *T. trichiura* infections. Kato-Katz detected 3/36 (8.33%) hookworm infections compared to mini-FLOTAC which detected 9/36 (25%) hookworm infections (Table 3.8.).

Table 3.8. The detection results for hookworm infections using the Kato-Katz thick smear and the mini-FLOTAC flotation with both ZnSO4 and NaCl solutions.

Parasite	Test	Positive	Negative	Total	Percent Positive
Hookworm	Kato-Katz	3	33	36	8.33
	mini-FLOTAC	9	27	36	25.00

For the detection rate comparison between the qPCR test and the mini-FLOTAC using both $ZnSO_4$ and NaCl solutions, no significant difference was found for hookworm infections (Table 3.9.). The group of comparative samples examined via qPCR and mini-FLOTAC were negative for both *A. lumbricoides* or *T. trichiura* infections. The qPCR test detected 5/28 (17.86%) hookworm infections compared to mini-FLOTAC which detected 8/28 (28.57%) hookworm infections (Table 3.9.).

Table 3.9. The detection results for hookworm infections using the quantitative PCR test and the mini-FLOTAC flotation with both ZnSO4 and NaCl solutions.

Parasite	Test	Positive	Negative	Total	Percent Positive
Hookworm	qPCR	5	23	28	17.86
	mini-FLOTAC	8	20	28	28.57

For the detection rate comparison between the NaCl and $ZnSO_4$ mini-FLOTAC solutions, no significant difference was found for hookworm infections (Table 3.10.). The group of comparative samples examined via both mini-FLOTAC solutions were negative for both *A. lumbricoides* or *T. trichiura* infections. The NaCl solution detected 13/47 (27.66%) hookworm infections compared to the $ZnSO_4$ solution which detected 10/47 (21.28%) hookworm infections (Table 3.10.).

Table 3.10. The detection results for hookworm infections using and the mini-FLOTAC NaCl and $ZnSO_4$ flotation solutions.

Parasite	mini-FLOTAC	Positive	Negative	Total	Percent Positive
Hookworm	NaCl	13	34	47	27.66
	$ZnSO_4$	10	37	47	21.28

3.3.5. The Impact of False-Negative Diagnostic Results

To evaluate the impact of the false-negative results provided by the MOH Kato-Katz test compared to the qPCR test, graphs were created using the R_o values for each STH, obtained from Brooker et al. (2006), to visualize the potential secondary infections produced by positive infections misclassified as false-negatives by Kato-Katz.

For hookworm infections, the R_o=2 and R_o=3 were calculated out to the 10^{th} generation for the 8 hookworm infections that were detected by qPCR but went undetected by the Kato-Katz test (Figure 3.8.). For *A. lumbricoides* infections, the R_o=2-5 were calculated out to the 10^{th} generation for the 2 *A. lumbricoides* infections that were detected by qPCR but went undetected by the Kato-Katz test (Figure 3.9.). For *T. trichiura* infections, the R_o=4-6 were calculated out to

the 10th generation for the 2 *T. trichiura* infections that were detected by qPCR but went undetected by the Kato-Katz test (Figure 3.10.).

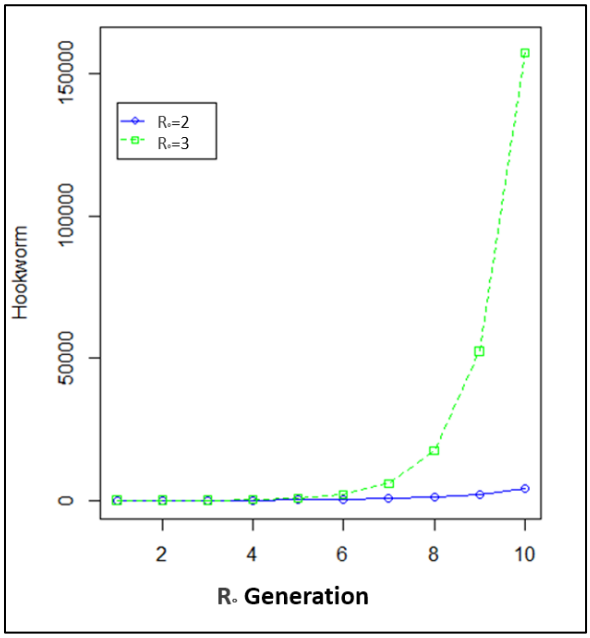

Figure 3.8. A graphic representation of the number of potential secondary infections, using a reproductive rate (R_o) of 2 and 3, produced from the 8 hookworm infections detected by the quantitative PCR test that went undetected using the Kato-Katz thick smear.

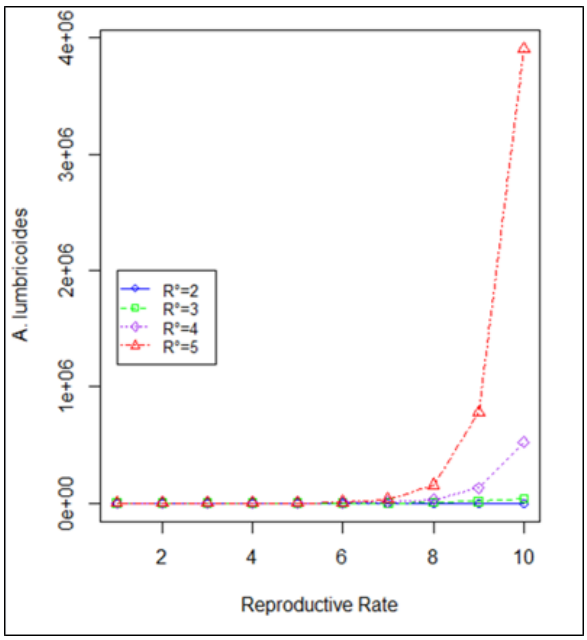

Figure 3.9. A graphic representation of the number of potential secondary infections, using a reproductive rate (R_o) of 2 and 3, produced from the 8 hookworm infections detected by the quantitative PCR test that went undetected using the Kato-Katz thick smear.

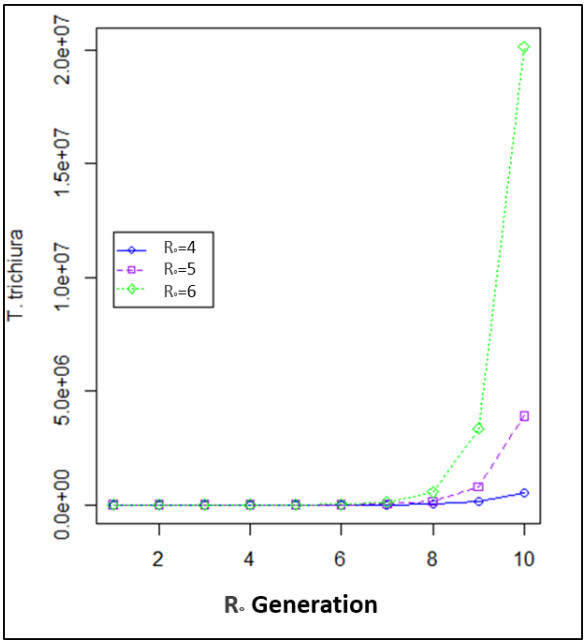

Figure 3.10. A graphic representation of the number of potential secondary infections, using a reproductive rate (R_o) of 2 and 3, produced from the 8 hookworm infections detected by the quantitative PCR test that went undetected using the Kato-Katz thick smear.

3.3.6. Prevalence By Community

When prevalence was evaluated by community, the predominant STH infection changed depending on the community examined. For the rural community of Maria Quiteria, hookworm had the highest prevalence with 23 infections (17.04%), and no participants were positive for either *A. lumbricoides* or *T. trichiura* (Table 3.11.). In the peri-urban community of CDGN, hookworm had the highest prevalence with 5 infections (7.25%). Both *A. lumbricoides* and *T. trichiura* had 1 infection each (1.45%) (Table 3.11.). For Queimadinha, the highest STH prevalence was for *A. lumbricoides* with 6 infections (7.89%), followed by 5 hookworm infections (6.58%) and 4 *T. trichiura* infections (5.26%) (Table 3.11.).

Table 3.11. Number of positive, total people sampled, and prevalence of soil-transmitted helminths in each study community. (Positive by ≥1 of the diagnostic tests)

Parasite	Maria Quiteria		CDGN		Queimadinha	
	Total	Prevalence	Total	Prevalence	Total	Prevalence
Ascaris lumbricoides	0	0	1	1.45	6	7.89
Hookworm	23	17.04	5	7.25	5	6.58
Trichuris trichiura	0	0	1	1.45	4	5.26

3.4. Discussion

To our knowledge, this study is the first to compare the Kato-Katz thick smear, the mini-FLOTAC flotation, and a multi-parallel real-time qPCR method to determine their relative abilities to detect STH infections in Brazil. In our three representative communities of Maria Quiteria, CDGN, and Queimadinha in Feira de Santana, Brazil, hookworm infection had the highest prevalence (11.11%), followed by *A. lumbricoides* infections (2.51%) and *T. trichiura* infections (1.79%). This is similar to reports from previous work in the rural area of Feira de Santana (Almeida et al. 2012), as well as other areas in Brazil (Pullan et al. 2008; Gonçalves et al. 2014; Monteiro et al. 2018). However, this differs from other Brazilian studies that demonstrated *A. lumbricoides* or *T. trichiura* to have higher prevalence than hookworm, such as in Salvador, Bahia (Mascarini-Serra et al. 2010), Itabuna, Bahia (Mariano et al. 2015). This prevalence difference is likely due to the focus on an urban center (Mascarini-Serra et al. 2010) or due to the sampling of young children ≤6 (Mariano et al. 2015).

This is one of only a few studies that sample the entire household for STH infection in Brazil. Studies that rely on sampling only pre-SAC and SAC in may provide inaccurate prevalence results that favor higher *A. lumbricoides* and *T. trichiura* prevalence (Anderson et al. 2015). This is because the heaviest-intensity *A. lumbricoides* and *T. trichiura* infections are found in children 5-15 years (Bethony et al. 2006), whereas typically the majority of hookworms are harbored by

adults >15 years (Anderson et al. 2015). The inclusion of the entire household in this study confirmed this, as the mean age of hookworm positive participants was 29.0 years. In fact, the mean age of every type of STH infection was found to occur in adulthood: for STH 29.03 years, for *A. lumbricoides* 22.29 years, and for *T. trichiura* 19.2 years. This demonstrates that adults act as a substantial reservoir for STH infections, contributing to continued transmission through egg-shedding within the environment and likely reinfection of the children in the household. If treatment programs were to continue following the WHO guidelines, treating children alone would have limited impact on transmission, particularly in areas of high hookworm prevalence (Anderson et al. 2015). To truly interrupt STH transmission in Feira de Santana and eliminate the disease in the entire population, the entire household must be included in STH surveillance and treated accordingly.

Our detection results demonstrated that all three diagnostic techniques detected hookworm infections. The lack of *A. lumbricoides* or *T. trichiura* detection by the mini-FLOTAC was due to absence of *A. lumbricoides* or *T. trichiura* infections in the samples analyzed by the method, not due to any false-negative results, as all samples negative for *T. trichiura* or *A. lumbricoides* using mini-FLOTAC were also negative using Kato-Katz and qPCR. The similar detection rates between Kato-Katz and mini-FLOTAC is in line with previous studies evaluating them both (B. Barda et al. 2013; Barda et al. 2014). However, the mini-FLOTAC provides several added benefits. The use of both the NaCl and $ZNSO_4$ flotation solutions detect protozoal parasites that cannot be detected using Kato-Katz. In addition, the time it takes to prepare and examine the mini-FLOTAC is about 18 minutes, compared to about 34-37 minutes for Kato-Katz (B. Barda et al. 2013). The similar detection rates between the qPCR test and Kato-Katz is likely due to the

relatively low number of direct sample comparisons evaluated between the two, and while not reaching significance, the qPCR test detected 6 more hookworm infections, 2 more *A. lumbricoides* infections, and 2 more *T. trichiura* infections than Kato-Katz. As previously demonstrated by Mejia et al. (2013) and Cimino et al. (2015), the qPCR test also allows for the detection of not only STH, but also *Strongyloides stercoralis*, *Giardia lamblia*, *Blastocystis hominis*, *Cryptosporidium parvum/hominis*, and *Entamoeba histolytica*. The qPCR test also allows for the differentiation of the hookworm species *A. duodenale* and *N. americanus*, which cannot be done by microscopic diagnostic techniques. The ability to correctly identify the predominant hookworm species is an important tool for developing a surveillance and response elimination program, since it will change the control approaches depending on the species. Since *N. americanus* cannot survive as long in the environment as *A. duodenale, N. americanus* transmission is frequently found at or in close proximity to defection sites (Hoagland and Schad 1978).

Alternatives to the traditional Kato-Katz have been pursued by numerous researchers (Speich et al. 2010; Levecke et al. 2011; B.D. Barda et al. 2013; Mejia et al. 2013). Alternative diagnostics are required because at low prevalence levels the Kato-Katz method is inadequate to regularly detect STH infections and differentiate the levels of infection intensity in the diminishing residual populations, and this inadequacy increases when only a single slide is examined. An improvement to the Kato-Katz is needed if Feira de Santana is to transition from its current morbidity control plan based on passive surveillance and toward an STH elimination plan based on an active surveillance and response system. The detection of STH infections in low prevalence areas is crucial for an effective surveillance and response system geared towards elimination.

While the qPCR test was unable to demonstrate a significant improvement in STH detection in this study, it did detect 6 hookworm infections, 2 *A. lumbricoides* infections, and 2 *T. trichiura* infections identified as false-negative via Kato-Katz and the potential populations deriving from these false-negatives can expand rapidly within a few generations. The qPCR test has previously demonstrated significantly higher sensitivity for *A. duodenale* and *T. trichiura* than the saline wet mount (Mejia et al. 2013) and significantly higher sensitivity for hookworm than either the Telemann concentration or McMaster diagnostic methods (Cimino et al. 2015). We posit that the reason for the lack of significance in this study is likely due to the low number of direct comparisons available between the two tests. An additional benefit of utilizing the qPCR test is that it allows for the detection of several additional gastrointestinal parasites with a single test. To achieve this using traditional microscopy would require several different tests. The qPCR provides a cost-saving approach compared to traditional microscopy, with a cost of <$1.00 per sample (Mejia et al. 2013) versus $2.60 per sample (Basuni et al. 2012) for microscopy, excluding the cost of a qPCR machine.

Previous estimates of STH infection in Brazil by Chammartin et al. (2014) have predicted infection risks of 6.0% for STH, 1.7% for hookworm, 3.6% for *A. lumbricoides,* and 1.4% for *T. trichiura*. This large-scale approach to modeling, while useful, underestimated the true prevalence of STH infections and does not provide the accuracy needed for an effective elimination program. In our study, we found a prevalence of 2.51% for *Ascaris lumbricoides*, 11.11% for hookworm, and 1.79% for *Trichuris trichiura*, in Feira de Santana, demonstrating higher hookworm prevalence that estimated using a large-scale approach. To implement an effective elimination program the differences in prevalence not only at the municipality-level but

at the community-level is needed. This study evaluated three representative communities, the rural community of Maria Quiteria, the peri-urban community of CDGN, and the urban community of Queimadinha. This allowed us to discern an ecologically stratified prevalence depending on community-type. The predominant infection-type found throughout the entire study area was hookworm, but this varied when individual communities were evaluated. Hookworm infections were the predominant type of STH found in both Maria Quiteria and CDGN, with Maria Quiteria having the highest hookworm prevalence of the three communities. Previous work in Maria Quiteria has also found hookworm to have the highest prevalence. (Almeida et al. 2012). The urban community of Queimadinha paints a different picture, with *A. lumbricoides* being the predominant type of STH infection, which is in line with the STH prevalence detected in another urban area of Bahia previously by Mascarini-Serra et. al. (2010).

The differing infection prevalence among communities demonstrates the importance of evaluating urban, peri-urban, and rural areas of a municipality separately, and tailoring the elimination strategy to fit the appropriate community. Different recommendations must account for the different biological requirements and routes of infection used by each STH. Hookworm transmission occurs through a fecal-cutaneous route, normally through larval penetration of bare feet. Since L3 are viable for only a few weeks in the environment (Jourdan et al. 2018), implementing a simple change such as footwear can contribute to a decrease in infection. *A. lumbricoides* and *T. trichiura* are transmitted fecal-orally, with ingestion of embryonated eggs from either contaminated food or hands. Infective eggs can survive for years (*A. lumbricoides*) or months (*T. trichiura*) (Jourdan et al. 2018). Thus, increased education on the need for proper hand- and food-washing measures can contribute to decreasing infection. Our study

demonstrates that the distribution of STH risk is not equivalent across an entire municipality. Therefore, implementation of control strategies should be optimized so that resource allocation focuses on geographical areas of high-infection risk, as opposed to uniformly distributing resources across the entire municipality.

3.5. Conclusion

STH infection risk is still high in many parts of the world. However, middle-income nations, such as Brazil, have achieved lower STH prevalence, and the time is right to replace the current morbidity control strategies with strategies aimed at STH elimination. Our study demonstrates that the qPCR diagnostic test is a valuable alternative to the current Kato-Katz diagnostic test in the low STH prevalence city of Feira de Santana, providing increased STH detection, decreased sample processing costs, and the ability to evaluate multiple gastrointestinal parasites with a single test. This study also demonstrates the importance of including entire households in STH sampling and of tailoring elimination strategies based on the different STH prevalence found in various communities. If the opportunity for elimination is to be achieved, we must employ more accurate diagnostics and adapt our sampling methodology to include all potential infections. The results of the current study provide support for adoption of one such alternative diagnostic technique and the implementation of different control approaches to shift the paradigm of STH control recommendations from morbidity control to elimination in city of Feira de Santana. Brazil.

3.6. References

Akuthota P, Weller PF. 2012. Eosinophilic pneumonias. Clin. Microbiol. Rev. 25:649–60. doi:10.1128/CMR.00025-12.

Almeida PHA de, Santana PCS, Silva AV da. 2012. Prevalência de protozoários e helmintos entéricos em residentes de são cristóvão, feira de santana, bahia, brasil. Arq. Ciênc. Saúde UNIPAR 16:61–66.

Anderson RM, Turner HC, Truscott JE, Hollingsworth TD, Brooker SJ. 2015. Should the Goal for the Treatment of Soil Transmitted Helminth (STH) Infections Be Changed from Morbidity Control in Children to Community-Wide Transmission Elimination? Aksoy S, editor. PLoS Negl. Trop. Dis. 9:e0003897. doi:10.1371/journal.pntd.0003897.

Arizono N, Yoshimura Y, Tohzaka N, Yamada M, Tegoshi T, Onishi K, Uchikawa R. 2010. Ascariasis in Japan: is pig-derived Ascaris infecting humans? Jpn. J. Infect. Dis. 63:447–8.

Assefa LM, Crellen T, Kepha S, Kihara JH, Njenga SM, Pullan RL, Brooker SJ. 2014. Diagnostic Accuracy and Cost-Effectiveness of Alternative Methods for Detection of Soil-Transmitted Helminths in a Post-Treatment Setting in Western Kenya. PLoS Negl. Trop. Dis. 8. doi:10.1371/journal.pntd.0002843.

Barda B, Cajal P, Villagran E, Cimino R, Juarez M, Krolewiecki A, Rinaldi L, Cringoli G, Burioni R, Albonico M. 2014. Mini-FLOTAC, Kato-Katz and McMaster: Three methods, one goal; Highlights from north Argentina. Parasites and Vectors 7:271. doi:10.1186/1756-3305-7-271.

Barda B, Zepherine H, Rinaldi L, Cringoli G, Burioni R, Clementi M, Albonico M. 2013. Mini-FLOTAC and Kato-Katz: helminth eggs watching on the shore of Lake Victoria. Parasit. Vectors 6:220. doi:10.1186/1756-3305-6-220.

Barda BD, Rinaldi L, Ianniello D, Zepherine H, Salvo F, Sadutshang T, Cringoli G, Clementi M, Albonico M. 2013. Mini-FLOTAC, an Innovative Direct Diagnostic Technique for Intestinal Parasitic Infections: Experience from the Field. PLoS Negl. Trop. Dis. 7:e2344. doi:10.1371/journal.pntd.0002344.

Basuni M, Mohamed Z, Ahmad M, Zakaria NZ, Noordin R. 2012. Detection of selected intestinal helminths and protozoa at Hospital Universiti Sains Malaysia using multiplex real-time PCR. Trop. Biomed. 29:434–42.

Basuni M, Muhi J, Othman N, Verweij JJ, Ahmad M, Miswan N, Rahumatullah A, Aziz FA, Zainudin NS, Noordin R. 2011. A pentaplex real-time polymerase chain reaction assay for detection of four species of soil-transmitted helminths. Am. J. Trop. Med. Hyg. 84:338–43. doi:10.4269/ajtmh.2011.10-0499.

Benício RA, da Silva GR, Fonseca MG. 2015. Anurans from a Caatinga area in state of Piauí, northeastern Brazil.

Bergquist R, Yang GJ, Knopp S, Utzinger J, Tanner M. 2015. Surveillance and response: Tools and approaches for the elimination stage of neglected tropical diseases. Acta Trop. 141:229–234. doi:10.1016/j.actatropica.2014.09.017.

Bethony J, Brooker S, Albonico M, Geiger SM, Loukas A, Diemert D, Hotez PJ. 2006. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. Lancet 367:1521–1532. doi:10.1016/S0140-6736(06)68653-4.

Booth M, Vounatsou P, N'goran EK, Tanner M, Utzinger J. 2003. The influence of sampling effort and the performance of the Kato-Katz technique in diagnosing Schistosoma mansoni and hookworm co-infections in rural Côte d'Ivoire. Parasitology 127:525–531. doi:10.1017/S0031182003004128.

Brazilian Ministry of Health. 2012. Integrated Strategic Action Plan for the Elimination fo Leprosy, Filariasis, Schistosomiasis and Onchocerciasis as a Public Health Problem, Trachoma as a Cause of Blindness and Control of Geohelmithiases: Action Plan 2011-2015. Brasilia, Brazil.

Brooker S. 2010. Estimating the global distribution and disease burden of intestinal nematode infections: adding up the numbers--a review. Int. J. Parasitol. 40:1137–44. doi:10.1016/j.ijpara.2010.04.004.

Brooker S, Clements ACA, Bundy DAP. 2006. Global Epidemiology, Ecology and Control of Soil-Transmitted Helminth Infections. Adv. Parasitol. 62:221–261. doi:10.1016/S0065-308X(05)62007-6.

Castelletti CHM, Silva JMC, Tabarelli M, Santos AMM. 2004. Quanto ainda resta da Caatinga? Uma estimativa preliminar.

Chaia G, Chaia AB, McAullife J, Katz N, Gasper D. 1968. Coprological diagnosis of schistosomiasis. II. Comparative study of quantitative methods. Rev. Inst. Med. Trop. Sao Paulo 10:349–353.

Chammartin F, Guimarães LH, Scholte RGC, Bavia ME, Utzinger J, Vounatsou P. 2014. Spatio-temporal distribution of soil-transmitted helminth infections in Brazil. Parasites and Vectors 7:440. doi:10.1186/1756-3305-7-440.

Cimino RO, Jeun R, Juarez M, Cajal PS, Vargas P, Echazú A, Bryan PE, Nasser J, Krolewiecki A, Mejia R. 2015. Identification of human intestinal parasites affecting an asymptomatic peri-urban Argentinian population using multi-parallel quantitative real-time polymerase chain reaction. Parasit. Vectors 8:380. doi:10.1186/s13071-015-0994-z.

Couto LD, Tibiriça SHCC, Pinheiro IO, Mitterofhe A, Lima AC, Castro MF, Gonçalves M, Silva MR, Guimarães RJPSPS, Rosa FM, et al. 2014. Neglected tropical diseases: prevalence and risk factors for schistosomiasis and soil-transmitted helminthiasis in a region of Minas Gerais State, Brazil. Trans. R. Soc. Trop. Med. Hyg. 108:363–71. doi:10.1093/trstmh/tru054.

Cringoli G. 2006. FLOTAC, a novel apparatus for a multivalent faecal egg count technique. Parassitologia 48:381–4.

Cutillas C, Callejón R, de Rojas M, Tewes B, Ubeda JM, Ariza C, Guevara DC. 2009. Trichuris suis and Trichuris trichiura are different nematode species. Acta Trop. 111:299–307. doi:10.1016/j.actatropica.2009.05.011.

Gonçalves AQ, Abellana R, Pereira-da-Silva HD, Santos I, Serra PT, Julião GR, Orlandi PP,

Ascaso C. 2014. Comparison of the performance of two spontaneous sedimentation techniques for the diagnosis of human intestinal parasites in the absence of a gold standard. Acta Trop. 131:63–70. doi:10.1016/j.actatropica.2013.11.026.

Hoagland KE, Schad GA. 1978. Necator americanus and Ancylostoma duodenale: Life history parameters and epidemiological implications of two sympatric hookworms of humans. Exp. Parasitol. 44:36–49. doi:10.1016/0014-4894(78)90078-4.

Jourdan PM, Lamberton PHL, Fenwick A, Addiss DG. 2018. Soil-transmitted helminth infections. Lancet 391:252–265. doi:10.1016/S0140-6736(17)31930-X.

Kato K, Miura M. 1954. Comparative examinations. Japanese J. Parasitol. 3:35.

Katz N, Chaves A, Pellegrino J. 1972. A simple device for quantitative stool thick-smear technique in Schistosomiasis mansoni. Rev. Inst. Med. Trop. Sao Paulo 14:397–400. doi:10.1590/S0036-46651999000200001.

Knopp S, Glinz D, Rinaldi L, Mohammed KA, N'Goran EK, Stothard JR, Marti H, Cringoli G, Rollinson D, Utzinger J. 2009. FLOTAC: A promising technique for detecting helminth eggs in human faeces. Trans. R. Soc. Trop. Med. Hyg. 103:1190–1194. doi:10.1016/j.trstmh.2009.05.012.

Knopp S, Mgeni AF, Khamis IS, Steinmann P, Stothard JR, Rollinson D, Marti H, Utzinger J. 2008. Diagnosis of soil-transmitted helminths in the era of preventive chemotherapy: Effect of multiple stool sampling and use of different diagnostic techniques. Engels D, editor. PLoS Negl. Trop. Dis. 2:e331. doi:10.1371/journal.pntd.0000331.

Levecke B, Behnke JM, Ajjampur SSR, Albonico M, Ame SM, Charlier J, Geiger SM, Hoa NTV, Ngassam RIK, Kotze AC, et al. 2011. A comparison of the sensitivity and fecal egg counts of the McMaster egg counting and Kato-Katz thick smear methods for soil-transmitted helminths. de Silva N, editor. PLoS Negl. Trop. Dis. 5:e1201. doi:10.1371/journal.pntd.0001201.

Liu C, Lu L, Zhang L, Bai Y, Medina A, Rozelle S, Smith DS, Zhou C, Zang W. 2017. More Poop, More Precision: Improving Epidemiologic Surveillance of Soil-Transmitted Helminths with Multiple Fecal Sampling using the Kato-Katz Technique. Am. J. Trop. Med. Hyg 97:870–875. doi:10.4269/ajtmh.16-0728.

Mariano APM, Santos EN, Santos TN dos, Mota TN, Silva JA da, Carvalho SMS, Silva MF da. 2015. Parasites in South Bahia: Focus on Giardiasis and Ascariasis among Preschoolers of Itabuna. Int. J. Heal. Sci. 3. doi:10.15640/ijhs.v3n1a4.

Martin LK, Beaver PC. 1968. Evaluation of Kato thick-smear technique for quantitative diagnosis of helminth infections.

Martins-Melo FR, Ramos AN, Alencar CH, Lima MS, Heukelbach J. 2017. Epidemiology of soil-

transmitted helminthiases-related mortality in Brazil. Parasitology 144:669–679. doi:10.1017/S0031182016002341.

Mascarini-Serra LM, Telles CA, Prado MS, Mattos SA, Strina A, Alcantara-Neves NM, Barreto ML. 2010. Reductions in the prevalence and incidence of geohelminth infections following a city-wide sanitation program in a Brazilian Urban Centre. PLoS Negl. Trop. Dis. 4:e588. doi:10.1371/journal.pntd.0000588.

McDonald JH. 2014. Handbook of Biological Statistics. 3rd ed. Baltimore: Sparky House Publishing.

Mejia R, Vicuña Y, Broncano N, Sandoval C, Vaca M, Chico M, Cooper PJ, Nutman TB. 2013. A novel, multi-parallel, real-time polymerase chain reaction approach for eight gastrointestinal parasites provides improved diagnostic capabilities to resource-limited at-risk populations. Am. J. Trop. Med. Hyg. 88:1041–1047. doi:10.4269/ajtmh.12-0726.

Monteiro KJL, Reis ERC Dos, Nunes BC, Jaeger LH, Calegar DA, Santos JP Dos, Maia A de O, Xavier SC das C, Bóia MN, Carvalho-Costa FA, et al. 2018. Focal persistence of soil-transmitted helminthiases in impoverished areas in the State of Piaui, Northeastern Brazil. Rev. Inst. Med. Trop. Sao Paulo 60:e24. doi:10.1590/s1678-9946201860024.

Morellato LPC, Haddad CFB. 2006. Introduction: The Brazilian Atlantic Forest. Biotropica 32:786. doi:10.1646/0006-3606(2000)032[0786:itbaf]2.0.co;2.

Pullan RL, Bethony JM, Geiger SM, Cundill B, Correa-Oliveira R, Quinnell RJ, Brooker S. 2008. Human helminth co-infection: Analysis of spatial patterns and risk factors in a Brazilian community. PLoS Negl. Trop. Dis. 2. doi:10.1371/journal.pntd.0000352.

de Silva NR, Brooker S, Hotez PJ, Montresor A, Engels D, Savioli L. 2003. Soil-transmitted helminth infections: updating the global picture. Trends Parasitol. 19:547–551. doi:10.1016/j.pt.2003.10.002.

Speich B, Knopp S, Mohammed KA, Khamis IS, Rinaldi L, Cringoli G, Rollinson D, Utzinger J. 2010. Comparative cost assessment of the Kato-Katz and FLOTAC techniques for soil-transmitted helminth diagnosis in epidemiological surveys. Parasit. Vectors 3:71. doi:10.1186/1756-3305-3-71.

Utzinger J, Rinaldi L, Lohourignon LK, Rohner F, Zimmermann MB, Tschannen AB, N'Goran EK, Cringoli G. 2008. FLOTAC: a new sensitive technique for the diagnosis of hookworm infections in humans. Trans. R. Soc. Trop. Med. Hyg. 102:84–90. doi:10.1016/j.trstmh.2007.09.009.

Veterinary Parasitology and Parasitic Diseases. Mini-FLOTAC technique-fresh faeces-HUMANS. Naples.

Weatherhead J, Cortés AA, Sandoval C, Vaca M, Chico M, Loor S, Cooper PJ, Mejia R. 2017.

Comparison of cytokine responses in ecuadorian children infected with Giardia, Ascaris, or both parasites. Am. J. Trop. Med. Hyg. 96:1394–1399. doi:10.4269/ajtmh.16-0580.

WHO. 1991. Basic laboratory methods in medical parasitology. WHO.

WHO. 1994. Bench aids for the diagnosis of intestinal parasites. Geneva: World Health Organization.

WHO. 1998. Guidelines for the evaluation of soil-transmitted helminthiasis and schistosomiasis at community level : a guide for managers of control programmes / A. Montresor ... [et al.]. Geneva.

WHO. 2004. Action against worms N°4: How to set up a deworming programme. Allen H, editor. Geneva: World Health Organization.

WHO. 2011. Helminth control in school-age children. A guide for managers of control programmes. 2nd ed. Geneva: World Health Organization.

Chapter 4. A Comparison of Household-Habitat Scale Ecological Niche Models Using WorldView-2, GeoEye-1, and Landsat 8 Satellite Products to Determine Ecological Distribution of Soil-Transmitted Helminth Infections in Three Representative Communities of Feira de Santana, Brazil

4.1. Introduction

Soil-transmitted helminth (STH) infections impact over 1.5 billion people worldwide (Pullan et al. 2014). An additional 3 billion people, the majority of whom are impoverished and live in tropical regions, are at risk for infection (Bethony et al. 2006). Three types of helminth parasite traditionally comprise STH: the hookworm species *Necator americanus* and *Ancylostoma duodenale*, the roundworm *Ascaris lumbricoides*, and the whipworm *Trichuris trichiura*.

STH geographic distribution is closely related to certain environmental and climatic conditions, such as higher temperatures and increased moisture, since these conditions are required to complete their life cycle (Bethony et al. 2006; Brooker et al. 2006; Mudenda et al. 2012). These constraints, combined with the impoverished condition of those infected, are the reason the majority of disease burden is found in the tropics (Bethony et al. 2006; Chammartin et al. 2014). While grouped under the umbrella term STH, each parasite has its own unique life cycle requirements, with different environments favoring the development of different STH parasites. For example, the ideal temperature for egg development varies by species, with 20-30°C the ideal temperature for hookworm, 28-32°C ideal temperature for *A. lumbricoides,* and 28-35°C ideal temperature for *T. trichiura* (Brooker et al. 2006; Scholte et al. 2012).

Current global efforts to combat the disease rely primarily on the World Health Organization (WHO) morbidity control strategy. This strategy focuses on using mass drug administration (MDA) in pre-school-aged children (Pre-SAC) and school-age children (SAC) either

annually or biannually depending on prevalence within a region (WHO 2012). Treatment consists of administering a benzimidazole, either albendazole or mebendazole, although doses of ivermectin can also be given as an alternative treatment for *A. lumbricoides* and *T. trichiura* (Jourdan et al. 2018). This treatment is intended to combat STH morbidity, but does not eliminate the disease, and reinfection is common due to the ubiquity of the infective STH eggs and larvae in the environment (Albonico et al. 1995).

An approach that transitions away from the current morbidity control efforts and towards STH elimination is needed, especially in areas considered low-risk. The WHO classifies low-risk as areas where STH prevalence amount SAC is <20% (WHO 2012). These low-risk areas provide an opportunity to enact this paradigm shift away from morbidity control and towards elimination, but require the creation of a targeted surveillance and response system that incorporates higher sensitivity diagnostic testing with high-resolution disease surveillance (Tambo et al. 2014; Bergquist et al. 2015).

Understanding the ecological niche of each STH parasite provides an opportunity to target a surveillance and response system through evaluation of the habitat distribution and environmental risk factors using remote sensing (RS) and geographic information systems (GIS). With the advent of very high-resolution (VHR) satellites, beginning with IKONOS in 1999, new avenues to evaluate species' niches within specific communities that focus on individual households and the surrounding habitat, have been opened. The new generation of VHR satellites provide spectral resolution of <5 m, compared to more widely used 15-30 m resolution satellites such as ASTER and LANDSAT 8, or the 1 km resolution satellites such as AVHRR and MODIS. In this study, we compared the ecological niches of STH in the city of Feira de Santana,

Brazil using the VHR satellites WorldView-2 (WV2) (DigitalGlobe Inc., Westminster, CO), that provides data from 9 multispectral (MS) bands, and GeoEye-1 (GE1) ((DigitalGlobe Inc., Westminster, CO), that provides data from 5 MS bands, as well as the high-resolution Landsat 8 satellite (National Aeronautics and Space Administration/U.S. Geological Survey, Greenbelt, MD), which provides data from 11 MS bands. VHR satellite resolution allows for multiple infections within a single house to be evaluated by offsetting the presence point to an adjacent pixel, which is not possible using Landsat 8 models because the pixels were too large (30 m). This means that using Landsat 8, a household with multiple positive infections are considered only a single "presence" point. Conversely, the use of VHR satellites allows for multiple household infections to be incorporated in the model, providing the ability to model prevalence instead of just presence/absence. Feira de Santana is located in the northeast Brazilian state of Bahia and STH prevalence has previously been estimated as low-risk (<20%) (Almeida et al. 2012; Chammartin et al. 2014). STH infections remain a problem and STH prevalence between communities within Feira de Santana can vary greatly, as previously demonstrated by Almeida et al. (2012).

To evaluate STH ecological niches within Feira de Santana, we used a software tool called maximum entropy species distribution (Maxent) modeling, which creates ecological niche models (ENMs) to accurately predict a species geographic distribution and ecological niche within an area (Phillips et al. 2006). Maxent creates ENMs by maximizes the entropy of a species distribution utilizing occurrence-only data and factoring in a set of environmental variable constraints for a species. The subsequent ENMs created not only provide an ecological niche map for the species, but also displays which environmental variable constraint(s) are most important in creating the ENM.

Previous research using RS and GIS tools to evaluate environmental conditions and risk factors conducive to STH in Brazil have provided a more information on STH ecology and epidemiology (Pullan et al. 2008; Barbosa et al. 2012; Mudenda et al. 2012; Scholte et al. 2012; Chammartin et al. 2013; Chammartin et al. 2014; Faria et al. 2017; Martins-Melo et al. 2017; Monteiro et al. 2018). However, there remains a paucity of data on STH geospatial distribution in Brazil, particularly on a city- or household-habitat scale, and this lack of an accurate assessment of STH geographical distribution is a major issue facing policy-makers and health providers (Brooker et al. 2006). Previous studies modeling STH in Brazil have either analyzed infection at the state- or country-scale, (Pullan et al. 2008; Mudenda et al. 2012; Scholte et al. 2012; Chammartin et al. 2014), or evaluated STH using only kernel density analysis, without assessing how environmental factors contribute to STH distribution and risk (Barbosa et al. 2012; Faria et al. 2017; Martins-Melo et al. 2017; Monteiro et al. 2018). While evaluating STH distribution on a state- or country-scale provides useful information, it does not provide the requisite resolution to effectively create a surveillance and response system geared towards STH elimination. Similarly, the evaluation of STH using kernel density analysis provides insight on infection cluster patterns and distributions within a community but provides no predictive analysis or extrapolation of STH infection either within that community or other similar communities. It also fails to account for the environmental factors that impact STH ecology and distribution within a given environment (Bethony et al. 2006; Mudenda et al. 2012; Chammartin et al. 2014).

In our study, the use of high-resolution Landsat 8 imagery, as well as VHR WV2 and GE1 imagery, enabled ENMs to be created for the city of Feira de Santana and three representative communities within Feira de Santana: the rural community of Maria Quiteria, the peri-urban

community of Campo Do Gado Novo (CDGN) and the urban community of Queimadinha. To create these ENMs, we used STH occurrence data collected in 2016 and a combination of biologically relevant vegetation, water, and soil variables.

Our main hypothesis was that the use of WV2 images would allow for the creation of more reliable and accurate ENMs than those created using GE1. This was posited because WV2's additional spectral bands allow for the evaluation of soil and the use of improved vegetation and water indices, while GE1 cannot be used to evaluate soil nor use the enhanced vegetation and water indices. A more complete understanding of what type of VHR images are required to effectively model STH distribution is important, especially considering the difference in expense between the two satellites.

Our second hypothesis was that using VHR satellite imagery would allow for effective and reliable STH ENM production evaluating both households and their surrounding environments, whereas the use of the coarser resolution satellite Landsat 8 would be unable to discern between households and their surrounding habitats. ENM accuracy at this very high-resolution-scale would allow the discernment between high and low risk areas within our three representative communities. This was posited because the VHR satellites resolution of <5 m enables the differentiation of the environment adjacent to the house, whereas the 30 m spatial and spectral resolution Landsat 8 provides encompasses too large an area per pixel to differentiate between households and their surrounding environment.

Our third hypothesis was that the ability to create very-high resolution (<5m) household-habitat ENMs for the three representative communities would reveal important environmental variables that reflect the ecological distribution of STH and that these would vary depending on

the type of community evaluated. As demonstrated in Chapter 3, as well as previously in Feira de Santana (Almeida et al. 2012), variation in both the prevalence and species of STH occurs between different communities within the same city. We propose that the ability to model on a scale of <5m and evaluate STH distribution differences between Feira de Santana communities is an important component for a successful elimination strategy.

4.2 Materials and Methods

4.2.1. Ethics Statement

Research protocols were approved by the Louisiana State University IRB #3633 (Appendix A.) and the Brazilian CONEP #54801816.0.1001.0053 (Appendix B.). Field and laboratory practices were reviewed and approved by the Feira de Santana Ministry of Health (MOH). All study participants were unpaid volunteers and agreed to participate with the stipulation that withdrawal could be had at any time. Oral explanations of the study were given to adults in the household, and participants were aware that positive cases would be treated by the MOH free of charge. Written consent was obtained from all participants, as well as child assent forms and guardian consent forms for children enrolled in the study (Appendices H., J., L.).

4.2.2. Study Area

The area of study was Feira de Santana, Brazil, a city in the northeastern Brazilian state of Bahia located at -39.040090, -12.192079 Decimal Degrees (DD) using the 1984 World Geodetic System (WGS 1984). Feira de Santana is the second largest city in Bahia, with a total area of 830.73 km^2 and a 2010 census population of 556, 642 and current 2108 population estimates are 609, 913 (IBGE-Instituto Brasileiro de Geografia e Estatística 2017). Feira de Santana has a tropical climate that alternates between sub-humid and sub-arid and is in an area that transitions

between the caatinga and Bahian coastal forest biomes. The annual mean temperature is 24° C, with an annual range of 22-27°C (Diniz and Galvani 2014). The year is divided into two precipitation seasons, a wet season and a dry season. The wet season, characterized increased humidity and increased precipitation, lasts from May through July and the dry season, characterized by low humidity and low levels of precipitation, lasts from August to March (Ministério da Agricultura Pecuária e Abastecimento; Diniz and Galvani 2014). Depending on the year, a second, short wet season may appear in December-January. The presence of a dry (Figure 4.1.A.) and wet (Figure 4.1.B.) season is associated with major changes in the local caatinga vegetation. Caatinga is composed of xeric scrublands and cacti, and vegetation density varies seasonally (Moraes; National Council of the Atlantic Forest Biosphere Reserve 2004; Mudenda et al. 2012). During the wet season, the scrubland vegetation is green and healthy, and during the dry season, vegetation cover and health sharply decreases.

This study focused on three representative communities in the Feira de Santana, an urban neighborhood, Queimadinha, a peri-urban neighborhood, CDGN, and a rural district, Maria Quiteria. These communities are official Brazilian Institute of Geography and Statistics (IBGE) census divisions and were chosen with the aid of Universidade Estadual do Feira de Santana (UEFS) collaborators and the Feira de Santana MOH as each area exemplifies one of three different types of communities comprising Feira de Santana.

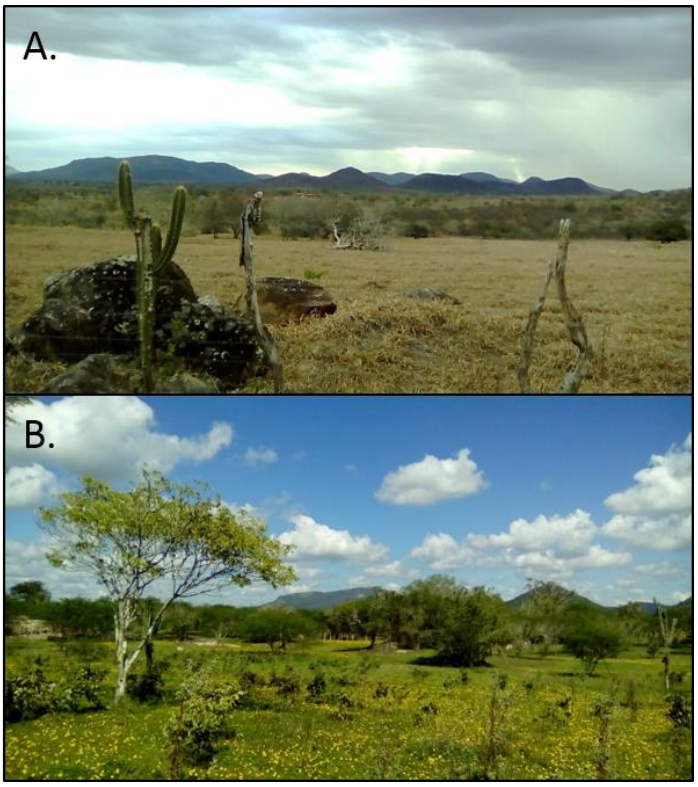

Figure 4.1. An example of the density variation of caatinga xeric scrubland vegetation in the same area during (A.) the dry season and (B.) the wet season.

4.2.3. Sampling Methodology

Individual household fecal sampling of 200 participants inhabitants was conducted across all study communities in conjunction with the Feira de Santana MOH personnel from May-November of 2016, with most of the sampling completed in October-November. Sample collection methodology followed established MOH practice of active sampling of households in the community in the morning until 12:00 pm, with sample cups left at the residence and retrieved the following dat. If the domicile was vacant, the next available household would be sampled. To obtain a spatially-distributed sample across each community, a different starting point in the community was chosen each day.

Maria Quiteria encompasses a larger area than the communities of CDGN and Queimadinha, and a major portion of this area is comprised of farmland interspersed with

sporadic housing sub-communities. To sample this area within the study sampling timeframe, an altered approach was taken where sampling originated at the local school and included the school children and their mothers. These were then traced back to the respective sub-community, and the surrounding households within the sub-community were sampled using the methodology described above.

Field GPS coordinates of each household and fecal sample collection was conducted in conjunction with Feira de Santana MOH personnel. Participants were interviewed to ascertain both household and individual participant socioeconomic information (Appendices D., F.). Fecal sample containers were left with participants and collected the next day for analysis at either the UEFS campus, the Feira de Santana MOH, or frozen at -20°C. Frozen samples were subsequently extracted at Universidade Federal da Bahia (UFBA), in Salvador, Brazil and transported back to the United States for PCR analysis in Houston, Texas.

4.2.4. Satellite Image Collection

Two different VHR satellites, GE1 and WV2, and one high-resolution satellite, Landsat 8, were used to obtain spectral images of Feira de Santana. These images were acquired with the aid of Dr. Jeffrey C. Luvall in accordance with the NASA NextView contract (NGA 2017). The GE1 satellite was launched in 2008 and provides a panchromatic resolution of up to 0.46 m and a multispectral resolution of up to 1.84 m (DigitalGlobe Inc. 2014). GE1 provides 5 different spectral bands: panchromatic, blue, green, red, and near infrared (NIR) (Table 4.1.). The WV2 satellite was launched in 2009 and provides up to 0.46 m panchromatic resolution and up to 1.84 m multispectral resolution (DigitalGlobe Inc. 2016). The WV2 satellite provides 9 different spectral bands, a panchromatic band and 8 multispectral bands. In addition to blue, green, red, and near

infrared, WV2 also provides 4 additional multispectral bands, coastal, yellow, red-edge, and near infrared-2 (NIR-2) (Table 4.1.). These added spectral bands enable additional environmental indices, such as a soil index, to be calculated (Wolf 2010). The high-resolution satellite Landsat 8 was launched in 2013 and consists of two different instruments, the Operational Land Imager (OLI) and the Thermal Infrared Sensor (TIRS) (Masek et al. 2019). These two sensors provide 11 different spectral bands, with up to a 15 m resolution for the panchromatic band, up to 30 m resolution for the 8 visible, NIR, and both short-wave infrared (SWIR) bands, and up to 100 m resolution for the two thermal infrared (TIR) bands (Table 4.1.).

Satellite images were obtained from GE1, WV2, and Landsat 8 as close to the months of October and November 2016 as possible. These two months were chosen since most of the field sampling was undertaken during this period. For GE1, 2 Standard LV2A images were obtained from December 10, 2016 (Table 4.2.). For WV2, 8 Standard LV2A images were obtained from November 7, 2016 (Table 4.2.). Both the GE1 and WV2 image sets had a pixel resolution of 2.0 m and were the best images available for Feira de Santana from the NASA NextView archive.

For Landsat 8, 1 L1TP image was obtained from August 3, 2016 (Table 4.2.). The resolution for the image was 30 m. A Landsat 8 image was chosen in August because all images from September-December 2016 had cloud coverage that obscured Feira de Santana.

4.2.5. Environmental Indices

The three RS satellites allow for the calculation of several different environmental indices, based on calculating the difference between various spectral bands reflectance or emittance values (Xue and Su 2017). To obtain environmental indices for Feira de Santana, we used ERDAS IMAGINE 2018 (Hexagon Geospatial, Madison, AL) software. Six different environmental indices

Table 4.1. A comparison of the GeoEye-1, WorldView-2, and LANDSAT 8 satellites spectral bands and their corresponding wavelength ranges.

GeoEye-1	Wavelength	Worldview-2	Wavelength	LANDSAT 8	Wavelength
Panchromatic	450-800 nm	Panchromatic	450-800 nm	Panchromatic	503-676 nm
		Coastal	400-450 nm	Coastal/Aerosol	435-451 nm
Blue	450-510 nm	Blue	450-510 nm	Blue	452-512 nm
Green	510-580 nm	Green	510-580 nm	Green	533-590 nm
		Yellow	585-625 nm	Red	636-673 nm
Red	655-690 nm	Red	630-690 nm	Near Infrared	851-879 nm
		Red Edge	705-745 nm	Shortwave Infrared 1	1566-1651 nm
Near Infrared 1	780-920 nm	Near Infrared 1	770-895 nm	Thermal Infrared 1	1060-1119 nm
				Thermal Infrared 2	1150-1251 nm
		Near Infrared 2	860-1040 nm	Shortwave Infrared 2	2107-2294 nm
				Cirrus	1363-1384 nm

Table 4.2. Acquisition information and number of GeoEye-1, WorldView-2, and Landsat 8 satellite images used to create environmental indices for Feira de Santana, Brazil.

Satellite	GeoEye-1	WorldView-2	Landsat 8
Sensor	GE01	WV02	OLI-TIRS
Product	Standard LV2A	Standard LV2A	L1TP
Number of Images	2	8	1
Acquisition Date	12/10/2016	11/7/216	8/3/2016
Acquisition Time (UTC)	12:50	12:57	12:42
Product Pixel Size	2.0 m	2.0 m	30.0 m

were calculated for WV2; 3 different environmental indices were calculated for GE1 and Landsat 8. Three types of indices were calculated: vegetation indices, water indices, and a soil index. These 3 indices were selected base on previously demonstrated positive associations with STH infection (Mabaso et al. 2003; Saathoff, Olsen, Sharp, et al. 2005; Mudenda et al. 2012; Schule et al. 2014). The 3 indices calculated for all satellite images were the normalized-difference vegetation index (NDVI) (Tucker 1979), soil-adjusted vegetation index (SAVI) (Huete 1988), and McFeeters normalized-difference water index (NDWI) (McFeeters 1996). In addition, WV2 allows for the calculation of three additional indices, modified versions of NDVI, NDWI, and the normalized-difference soil index (NDSI) (Deng et al. 2015). These modified indices are the

WorldView improved vegetation Index (WVVI) and the WorldView water index (WVWI), and the WorldView soil index (WVSI) (Wolf 2010).

The NDVI (1), is the most widely used remote sensing vegetation index (Glenn et al. 2008; Xue and Su 2017). The index is based on analyzing the difference in absorption contrast between the red spectral band, that is readily absorbed by chlorophylls a and b, and the NIR spectral band, which is strongly reflected by plant cell walls (Glenn et al. 2008). This is useful in distinguishing vegetation cover from water and other land cover classes.

The SAVI (2) was proposed by Huete (1988) to adjust NDVI values to more accurately capture vegetation by implementing a correction factor for soil substrate variations. These variations impact reflectance values and SAVI corrects this by normalizing the reflectance values of the NIR and red bands using an adjustment factor (L=0.5) to reduce soil reflectance.

McFeeters NDWI (3) was proposed by McFeeters (1996) for use in identifying surface water in non-urban areas. McFeeters NDWI is calculated using the green and NIR spectral bands, with the green band maximizing the green reflectance of water bodies compared to the minimization of NIR reflectance from those water bodies. NDWI also takes advantage of the high NIR reflectance of vegetation and soil, thereby highlighting water bodies (Li et al. 2013).

The WVVI (4), WVWI (5), and WVSI (6) are modifications of NDVI, McFeeters NDWI, and NDSI that take advantage of the added coastal band, yellow band, and NIR2 band provided by the WV2 satellite (Wolf 2010; Wolf 2012). WVVI uses the NIR2 band instead of the traditional broad NIR band for vegetation, with the higher reflectance values of NIR2 allowing greater difference between absorbed red and reflected NIR2 to produce more accurate positive vegetation classification. WVWI replaces the NDWI green band with the WV2 coastal band and

the NDWI NIR band with the WV2 NIR2 band, allowing for a greater difference between the low coastal band reflectance and NIR2 high reflectance values. This provides a more discrete threshold for discerning water bodies, and decreases the chance of false positive detections (Wolf 2010). The WVSI band uses the green and yellow bands in place of the usual short-wave infrared (SWIR) and near-infrared bands used to differentiate soil reflectance. The unique difference between the green and yellow bands soil reflectance allows for the detection of soil content without a SWIR band.

$$NDVI = (NIR - Red) / (NIR + Red) \quad (1)$$

$$SAVI = [(NIR-Red) / (NIR + red + L)] \times (1 + L) \quad (2)$$

$$NDWI = (Green-NIR) / (Green + NIR) \quad (3)$$

$$WVVI = (NIR2 - Red) / (NIR2 + Red) \quad (4)$$

$$WVWI = (Coastal - NIR2) / (Coastal + NIR2) \quad (5)$$

$$WVSI = (Green - Yellow) / (Green + Yellow) \quad (6)$$

4.2.6. Maximum Entropy Species Distribution Modeling and ENMTools Software

To create ecological niche models (ENMs) demonstrating predicted STH niche distribution and the corresponding environmental risk factors, Maximum Entropy Species Distribution Modeling (Maxent) software V. 3.4.1., was used (Phillips et al.). Maxent is a species distribution modeling (SDM) tool that utilizes occurrence-only data to estimate the distribution of a species (Phillips et al. 2006). Maxent estimates species distribution in a given space by determining which distribution has the maximum amount of entropy while using environmental values at occurrence points as model constraints (Phillips et al. 2017). The models underlying maximum entropy principle indicates that out of the numerous distribution models created that satisfy

these constraints, the one that with maximum entropy, or closest to uniform, be chosen. This approach is equivalent determining the maximum likelihood Gibbs distribution, or an exponential distribution in a linear combination of features (Phillips et al. 2004).

To interpret the ENM outputs created by Maxent, we evaluated the area under the receiver operating curve (ROC) (AUC). The ROC is a plot of the true positive rate against the false positive rate for a specific threshold parameter, displaying the performance of a particular classifier dependent on that threshold parameter (Phillips et al. 2004). ENM AUC values are used to interpret model validity and reliability, with an AUC of 1 indicating a perfect model and an AUC of 0.5 signifying the model behaves no better than chance. ENMs with AUC values >0.9 are considered very good models, those between 0.7-0.9 are considered good models, and those <0.7 are considered uninformative models (Swets 1998; Baldwin 2009). Maxent also provides variable contribution tables and Jackknife tests that demonstrate the importance of each input variable towards the ENM itself (Elith et al. 2011).

An additional statistical explanation of Maxent more recently described is that it behaves as an inhomogeneous Poisson process (IPP) (Fithian and Hastie 2013), allowing for the modeling of probability of presence in addition to modeling estimated geographic range. To model this probability of presence, Maxent has now incorporated a complementary log-log (cloglog) transformation in its modeling software:

$$\text{Probability of presence} = 1 - \exp(-\exp(H)p_{\lambda}(z)) \cdot$$ (Phillips et al. 2017)

The ENMTools software (Warren et al. 2010) enables additional analysis of Maxent ENMs beyond the tradition evaluation of model AUC values. While AUC values provide useful model information, they have shortcomings (Lobo et al. 2008; Yackulic et al. 2013). ENMTools provides

an alternative method of model performance using the Akaike information criterion (AIC) (Akaike 1974), which ranks models on their amount of discrepancy from the true probability distribution, with the best performing models having the lowest AIC (Warren and Seifert 2011). To evaluate model performance, this study utilized the sample size corrected AIC score, known as AICc.

4.2.7. Satellite Image Processing and Analysis

A schematic for WV2, GE1, and Landsat 8 image processing and analysis was constructed (Figure 4.2.) to created Maxent ENMs and evaluate model performance. A total of four study areas, Queimadinha, CDGN, Maria-Quiteria-1, and Maria Quiteria-2, from the three representative communities, were evaluated (Figure 4.3.). The first step was to mosaic the WV2 and GE1 images to create a single, comprehensive image of Feira de Santana. The mosaic process blends several separate radiometric geocoded images to create a single, balanced radiometric image (Inampudi 1998). Image mosaics were created using ERDAS IMAGINE 2018 Version 16.5 (Hexagon Geospatial Madison, AL) for the 8 WV2 images and the 2 GE1 images used.

To obtain environmental indices, the ERDAS IMAGINE 2018 Version 16.5 (Hexagon Geospatial Madison, AL) Raster Indices function was used to calculate NDVI, SAVI, NDWI, WVVI, WVWI, and WVSI for all three satellite images. The indices were imported into ArcMap 10.6 (ERSI Redlands, CA) and clipped to the study area boundaries.

Images for each study area were adjusted to exclude cloud cover and encompass as much area overlap between the satellite products as possible. Cloud cover was excluded because it interferes with remote sensors and limits the amount of valid land surface spectral reflectance that can be obtained (Sun et al. 2017). This in turn limits the ability to accurately run classification algorithms such as environmental indices (Sedano et al. 2011).

In the CDGN community, a single large cloud formation was clipped out of the image, and the total area evaluated was 1.61 km^2. Two different segments of Maria Quiteria were evaluated, a smaller area labeled Maria Quiteria-1, and a larger area labeled Maria Quiteria-2. Maria Quiteria-1, with an area of 0.85 km^2, is covered by the WV2, GE, and Landsat 8 satellite products which allows direct comparisons between them. Maria Quiteria-2, with an area of 7.53 km^2, was evaluated using only the GE1 and Landsat 8 satellites, since the cloud cover of the WV2 image obscured the majority of the Maria Quiteria-2 region, including the households surveyed. For the Queimadinha community, a single cloud was clipped out of the census tract, ID:291080005080113 (IBGE). This single census tract, with an area of 0.042 km^2, was the area of the community evaluated because it contained the majority of household sampled, and all STH positive households. Due to an outbreak of civil unrest within the community, we were unable to survey the rest of Queimadinha.

Maxent modeling cannot account for a quantitative measurement at a given coordinate point, such as multiple infections within the same household, and therefore counts them all as a single occurrence. To account for households with multiple infected participants, geolocated points were offset to an adjacent pixel (2.00 m adjustment) of similar value for GE1 and WV2. This allowed for the number infected per household to be weighed in the analysis. Point offsetting could not be conducted for Landsat 8, as the pixel size (30 m x 30 m) is too large. Adjusting points to the nearest pixel would lead to inaccurate model results. All coordinate tables were then converted into comma-separated values files and combined with the ASCII environmental layers to produce ENMs.

Comparison of satellite ENMs followed a two-step process: the first step compared WV2 and GE1 ENMs using AUC and AIC_c values to determine which satellite products created the best performing models; the second step compared the best performing VHR satellite ENMs to Landsat 8 ENMs using the ENM AUC values (Figure 4.3.). To decrease the total number of ENMs run, Pearson correlation coefficient (r) matrices for WV2, GE1, and Landsat 8 environmental indices were created to detect collinear variables (Dormann et al. 2013; Midzi et al. 2018). For the WV2 indices, NDVI, SAVI, and WVVI and WVWI and NDWI were highly correlated across all 4 areas with $r \geq 0.8$. For both GE1 and Landsat 8, NDVI and SAVI were highly correlated across all 4 areas $r \geq 0.8$. These colinear variables were excluded from our model combinations to reduce model uncertainty and increase model performance.

A total of 22 ENM variable combinations were created for WV2 (Table 4.3). For both GE1 (Table 4.4.) and Landsat 8 (Table 4.5.), 5 ENM variable combinations were created. These combinations were run for each of the four study areas, evaluating the presence of any STH as well as each STH parasite separately. To assess AUC values for of each model, the cloglog Maxent transformation was used (Phillips et al. 2017). Due to the low number of presence points in each area, only Maxent's training AUC was evaluated (Phillips et al. 2004). To rank ENMs based on AIC_c using ENMTools, only informative models with AUC values>0.7 were included in analysis. Maxent's raw output of each ENM was input into ENMTools and for Landsat 8, duplicate household points were removed (Warren and Seifert 2011). The ENM(s) with the lowest AIC_c were considered the best performing model(s). These were imported into ArcMap 10.6 (ESRI, Redlands, CA) for map creation. To determine the most important environmental variables for

the best performing ENMs, Maxent's percent contribution tables and Jackknife tests of variable importance were evaluated (Phillips 2008).

For VHR satellite comparison model performance was determined using AIC_c. AIC_c scores were used because AUC values can be problematic when attempting to predict potential species distribution (Peterson et al. 2008; Jiménez-Valverde 2012). For the comparison of the best performing VHR satellite and Landsat 8, model performance was based on evaluating AUC values because AIC analysis is not applicable for ENMs with different sample sizes.

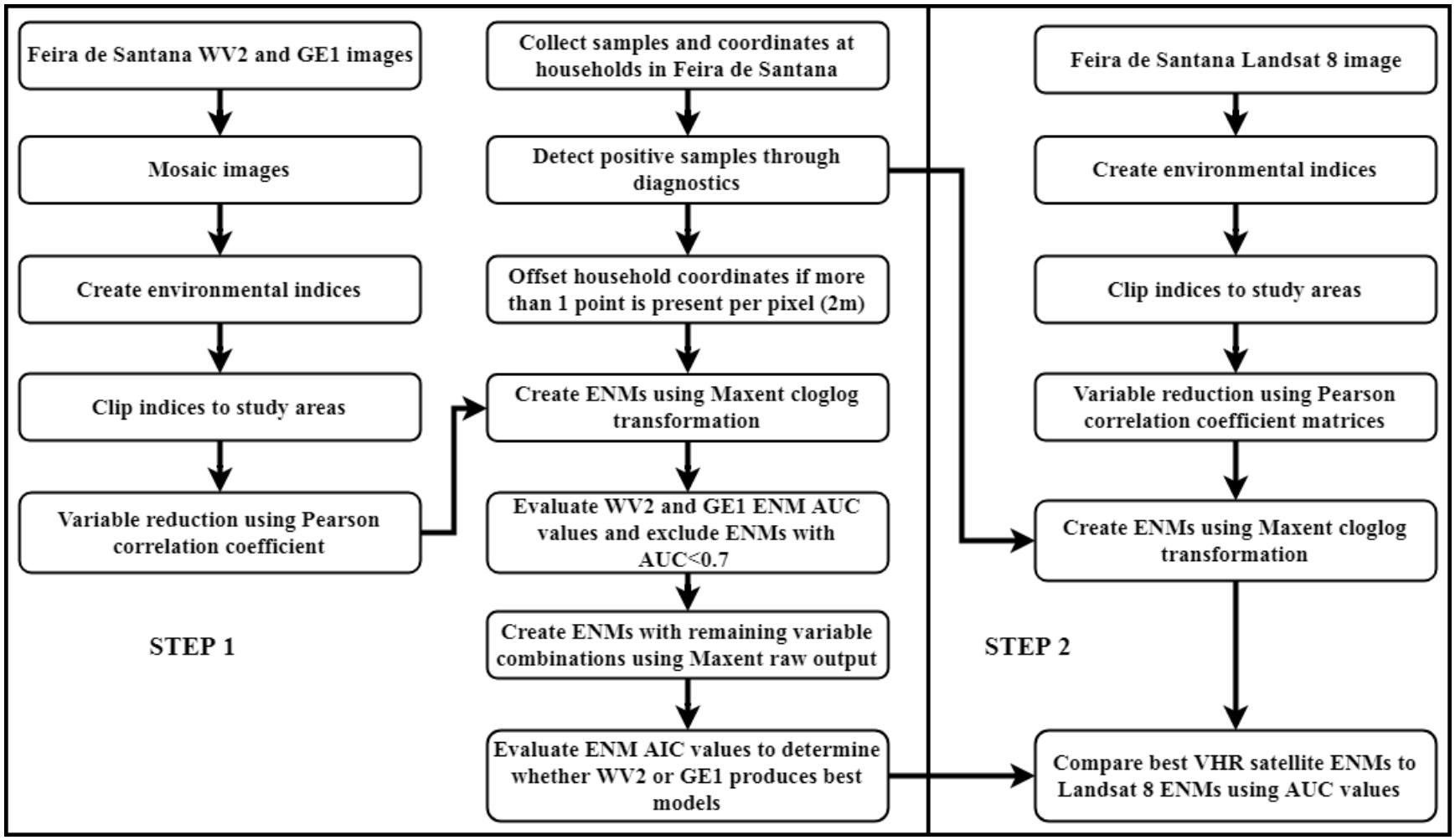

Figure 4.2. Schematic for creation and comparison of soil-transmitted helminth ecological niche models (ENMs) in the study areas of Feira de Santana, Brazil using two very high-resolution (VHR) satellites WorldView-2 (WV2) and GeoEye-1 (GE1), and the Landsat 8 satellite.

(AUC=Area Under the Curve, AIC=Akaike Information Criterion)

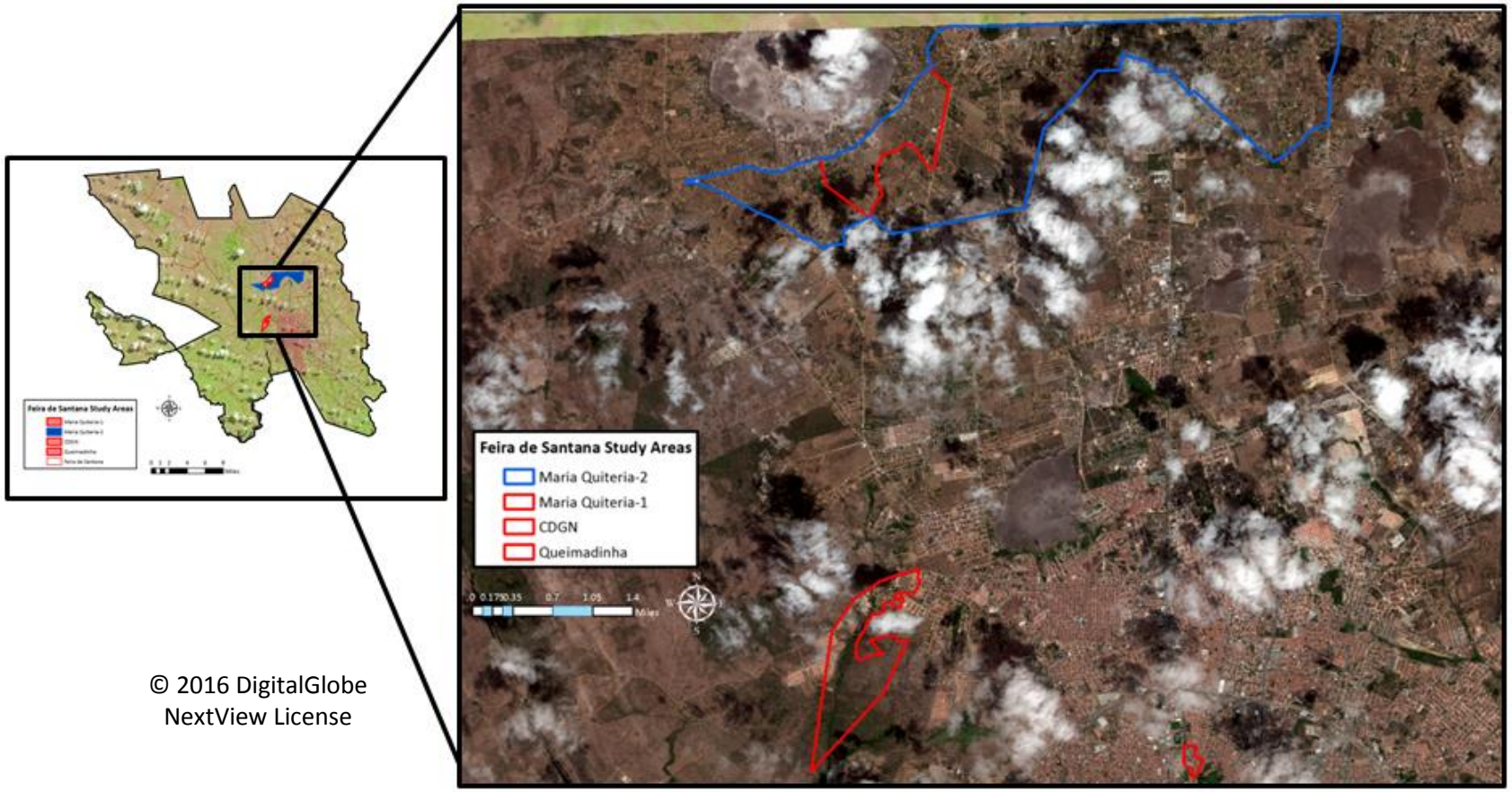

Figure 4.3. Map of Feira de Santana, Brazil and the four study areas (left). Close-up view of the four study areas of Queimadinha, Campo Do Gado Novo (CDGN), Maria Quiteria-1, and MQ-2 (right). (*GeoEye-1 satellite image used on right*)

Table 4.3. The 22 WorldView-2 model combinations of vegetation, water, and soil indices used to evaluate soil-transmitted helminths in Feira de Santana, Brazil

Model	Vegetation Index	Water Index	Soil Index
1	NDVI	NDWI	WVSI
2	SAVI	NDWI	WVSI
3	WVVI	NDWI	WVSI
4	NDVI	WVWI	WVSI
5	SAVI	WVWI	WVSI
6	WVVI	WVWI	WVSI
7	NDVI	NDWI	Omitted
8	NDVI	Omitted	WVSI
9	Omitted	NDWI	WVSI
10	SAVI	NDWI	Omitted
11	SAVI	Omitted	WVSI
12	NDVI	WVWI	Omitted
13	Omitted	WVWI	WVSI
14	SAVI	WVWI	Omitted
15	WVVI	WVWI	Omitted
16	WVVI	Omitted	WVSI
17	NDVI	Omitted	Omitted
18	SAVI	Omitted	Omitted
19	Omitted	NDWI	Omitted
20	WVVI	Omitted	Omitted
21	Omitted	WVWI	Omitted
22	Omitted	Omitted	WVSI

Table 4.4. The 5 GeoEye-1 model combinations of vegetation and water indices used to evaluate soil-transmitted helminths in Feira de Santana, Brazil.

Model	Vegetation Index	Water Index
1	NDVI	NDWI
2	SAVI	NDWI
3	NDVI	Omitted
4	SAVI	Omitted
5	Omitted	NDWI

Table 4.5. The 5 Landsat 8 model combinations of vegetation and water indices used to evaluate soil-transmitted helminths in Feira de Santana, Brazil.

Model	Vegetation Index	Water Index
1	NDVI	NDWI
2	SAVI	NDWI
3	NDVI	Omitted
4	SAVI	Omitted
5	Omitted	NDWI

4.3. Results

4.3.1. Prevalence Across Households

Across the three areas of Feira de Santana evaluated geospatially, a total of 63 houses comprised of 200 total participants were sampled. Of those 63 houses, 21 were positive for at least one STH infection (33.33%) (Table 4.6.) Seventeen households were positive for hookworm infection (26.98%), 6 households were positive for *A. lumbricoides* infection (9.52%), and 5 households were positive for *T. trichiura* infection (7.94%) (Table 4.6.). Of the 200 participants evaluated, 26 were positive for at least one STH infection (13%), 21 participants had hookworm infections (10.5%), 7 participants had *A. lumbricoides* infections (3.5%), and 5 participants had *T. trichiura* infections (2.50%) (Table 4.6.).

Table 4.6. The total number of houses and people sampled in Feira de Santana and the positive number and prevalence of any soil-transmitted helminth, hookworm, *Ascaris lumbricoides*, and *Trichuris trichiura* found in houses and people in Queimadinha, Campo do Gado Novo, and Maria Quiteria.

	Houses	Prevalence	People	Prevalence
Number Sampled	63		200	
Soil-transmitted helminths	21	33.33	26	13.00
Hookworm	17	26.98	21	10.50
Ascaris lumbricoides	6	9.52	7	3.50
Trichuris trichiura	5	7.94	5	2.50

4.3.2. Comparison of WorldView-2 and GeoEye-1 Ecological Niche Models Using Area Under the Curve (AUC) Values

As our first step in comparing the two VHR satellites WV2 and GE1, ENMs using WV2 and GE1 satellite images of Queimadinha, CDGN, and Maria Quiteria-1 were created and all ENM AUC values were compared. For the area Maria Quiteria-2 only GE1 ENM AUCs were compared since ENMs using WV2 could not be produced.

In Queimadinha, 27 ENMs evaluating STH, hookworm, *A. lumbricoides*, and *T. trichiura* were produced using the VHR satellites for a total of 108 ENMs. For STH, 9 presence points were

used and the ENMs with the highest AUC values were WV2 model 9 (NDWI, NDVI), WV2 model 13 (WVWI, WVSI), and WV2 model 22 (WVSI), with model AUCs=0.781 (Table 4.7.). For hookworm, 5 presence points were used and the ENMs with the highest AUC values were WV2 model 9 (NDWI, NDVI), WV2 model 13 (WVWI, WVSI), and WV2 model 22 (WVSI), with model AUCs=0.805 (Table 4.8.). For *A. lumbricoides*, 6 presence points were used and the ENMs with the highest AUC values were WV2 model 9 (NDWI, NDVI), WV2 model 13 (WVWI, WVSI), and WV2 model 22 (WVSI) (Table 4.9.), with model AUCs=0.785. For *T. trichiura*, 4 presence points were used and only 3 ENMs produced AUC values >0.7. The 3 ENMs were WV2 model 9 (NDWI, NDVI), WV2 model 13 (WVWI, WVSI), and WV2 model 22 (WVSI) (Table 4.10.), with model AUCs=0.751.

In CDGN, 27 ENMs evaluating STH and hookworm were produced using the VHR satellites for a total of 54 ENMs. *A. lumbricoides* and *T. trichiura* could not be evaluated because each only had a single presence point in CDGN. For STH, 6 presence points were used and the highest ENM AUC was for GE1 CDGN model 5 (NDWI) with an AUC=0.921 (Table 4.11). For hookworm, 5 presence points were used and the highest ENM AUCs were for GE1 CDGN model 1 (NDWI, NDVI), GE1 CDGN model 2 (SAVI, NDWI), and GE1 CDGN model 5 (NDWI) with an AUC=0.922 (Table 4.11.).

In Maria Quiteria-1, 27 total ENMs were created to evaluate hookworm using the VHR satellites. Hookworm infections were the only STH detected in this area, and so were the only STH evaluated. For hookworm ENMs, 5 presence points were used and the highest ENM AUCs were for GE1 Maria Quiteria-1 model 1 (NDVI, NDWI) and GE1 Maria Quiteria-1 model 2 (SAVI, NDWI) with an AUC=0.820 (Table 4.12.).

Table 4.7. GeoEye-1 and WorldView-2 model AUC values for soil-transmitted helminths (STH) in Queimadinha.

Parasite	Satellite/Area	Model Number	AUC
STH	WV2 Queimadinha	9	0.781
STH	WV2 Queimadinha	13	0.781
STH	WV2 Queimadinha	22	0.781
STH	WV2 Queimadinha	16	0.749
STH	WV2 Queimadinha	3	0.748
STH	WV2 Queimadinha	6	0.748
STH	WV2 Queimadinha	1	0.742
STH	WV2 Queimadinha	2	0.742
STH	WV2 Queimadinha	4	0.742
STH	WV2 Queimadinha	5	0.742
STH	WV2 Queimadinha	8	0.742
STH	WV2 Queimadinha	11	0.742
STH	GE1 Queimadinha	1	0.726
STH	GE1 Queimadinha	2	0.726
STH	GE1 Queimadinha	3	0.726
STH	GE1 Queimadinha	4	0.726
STH	WV2 Queimadinha	7	0.706
STH	WV2 Queimadinha	10	0.706
STH	WV2 Queimadinha	12	0.693
STH	WV2 Queimadinha	14	0.693
STH	WV2 Queimadinha	17	0.688
STH	WV2 Queimadinha	18	0.688
STH	WV2 Queimadinha	15	0.660
STH	WV2 Queimadinha	20	0.660
STH	GE1 Queimadinha	5	0.557
STH	WV2 Queimadinha	19	0.500
STH	WV2 Queimadinha	21	0.500

Table 4.8. GeoEye-1 and WorldView-2 model AUC values for hookworm in Queimadinha.

Parasite	Satellite/Area	Model Number	AUC
Hookworm	WV2 Queimadinha	9	0.805
Hookworm	WV2 Queimadinha	13	0.805
Hookworm	WV2 Queimadinha	22	0.805
Hookworm	GE1 Queimadinha	1	0.801
Hookworm	GE1 Queimadinha	2	0.801
Hookworm	GE1 Queimadinha	3	0.801
Hookworm	GE1 Queimadinha	4	0.801
Hookworm	WV2 Queimadinha	3	0.789
Hookworm	WV2 Queimadinha	6	0.789
Hookworm	WV2 Queimadinha	16	0.789
Hookworm	WV2 Queimadinha	1	0.738
Hookworm	WV2 Queimadinha	2	0.738
Hookworm	WV2 Queimadinha	4	0.738
Hookworm	WV2 Queimadinha	5	0.738
Hookworm	WV2 Queimadinha	8	0.738
Hookworm	WV2 Queimadinha	11	0.738
Hookworm	WV2 Queimadinha	7	0.660
Hookworm	WV2 Queimadinha	10	0.660
Hookworm	WV2 Queimadinha	12	0.660
Hookworm	WV2 Queimadinha	14	0.660
Hookworm	WV2 Queimadinha	17	0.660
Hookworm	WV2 Queimadinha	18	0.660
Hookworm	WV2 Queimadinha	15	0.590
Hookworm	WV2 Queimadinha	20	0.590
Hookworm	WV2 Queimadinha	19	0.500
Hookworm	WV2 Queimadinha	21	0.500
Hookworm	GE1 Queimadinha	5	0.500

Table 4.9. GeoEye-1 and WorldView-2 model AUC values for *Ascaris lumbricoides* in Queimadinha.

Parasite	Satellite/Area	Model Number	AUC
A. lumbricoides	WV2 Queimadinha	9	0.785
A. lumbricoides	WV2 Queimadinha	13	0.785
A. lumbricoides	WV2 Queimadinha	22	0.785
A. lumbricoides	WV2 Queimadinha	3	0.777
A. lumbricoides	WV2 Queimadinha	6	0.777
A. lumbricoides	WV2 Queimadinha	16	0.777
A. lumbricoides	WV2 Queimadinha	1	0.764
A. lumbricoides	WV2 Queimadinha	2	0.764
A. lumbricoides	WV2 Queimadinha	4	0.764
A. lumbricoides	WV2 Queimadinha	5	0.764
A. lumbricoides	WV2 Queimadinha	8	0.763
A. lumbricoides	WV2 Queimadinha	11	0.763
A. lumbricoides	WV2 Queimadinha	7	0.749
A. lumbricoides	WV2 Queimadinha	10	0.749
A. lumbricoides	WV2 Queimadinha	12	0.740
A. lumbricoides	WV2 Queimadinha	14	0.740
A. lumbricoides	WV2 Queimadinha	15	0.738
A. lumbricoides	WV2 Queimadinha	20	0.731
A. lumbricoides	WV2 Queimadinha	17	0.719
A. lumbricoides	WV2 Queimadinha	18	0.719
A. lumbricoides	GE1 Queimadinha	1	0.716
A. lumbricoides	GE1 Queimadinha	2	0.716
A. lumbricoides	GE1 Queimadinha	3	0.715
A. lumbricoides	GE1 Queimadinha	4	0.715
A. lumbricoides	WV2 Queimadinha	19	0.500
A. lumbricoides	WV2 Queimadinha	21	0.500
A. lumbricoides	GE1 Queimadinha	5	0.500

Table 4.10. GeoEye-1 and WorldView-2 model AUC values for *Trichuris trichiura* in Queimadinha.

Parasite	Satellite/Area	Model Number	AUC
T. trichiura	WV2 Queimadinha	9	0.751
T. trichiura	WV2 Queimadinha	13	0.751
T. trichiura	WV2 Queimadinha	22	0.751
T. trichiura	GE1 Queimadinha	1	0.681
T. trichiura	GE1 Queimadinha	2	0.681
T. trichiura	WV2 Queimadinha	3	0.669
T. trichiura	WV2 Queimadinha	6	0.669
T. trichiura	WV2 Queimadinha	16	0.669
T. trichiura	WV2 Queimadinha	15	0.649
T. trichiura	WV2 Queimadinha	20	0.649
T. trichiura	WV2 Queimadinha	2	0.641
T. trichiura	WV2 Queimadinha	5	0.641
T. trichiura	WV2 Queimadinha	8	0.641
T. trichiura	WV2 Queimadinha	11	0.641
T. trichiura	GE1 Queimadinha	3	0.641
T. trichiura	GE1 Queimadinha	4	0.641
T. trichiura	WV2 Queimadinha	1	0.639
T. trichiura	WV2 Queimadinha	4	0.639
T. trichiura	WV2 Queimadinha	7	0.620
T. trichiura	WV2 Queimadinha	10	0.620
T. trichiura	WV2 Queimadinha	12	0.620
T. trichiura	WV2 Queimadinha	14	0.620
T. trichiura	WV2 Queimadinha	17	0.620
T. trichiura	WV2 Queimadinha	18	0.620
T. trichiura	WV2 Queimadinha	19	0.500
T. trichiura	WV2 Queimadinha	21	0.500
T. trichiura	GE1 Queimadinha	5	0.500

Table 4.11. GeoEye-1 and WorldView-2 model AUC values for STH in Campo Do Gado Novo (CDGN).

Parasite	Satellite/Area	Model Number	AUC
STH	GeoEye-1 CDGN	5	0.921
STH	GeoEye-1 CDGN	1	0.919
STH	GeoEye-1 CDGN	2	0.919
STH	WorldView-2 CDGN	3	0.853
STH	WorldView-2 CDGN	6	0.853
STH	WorldView-2 CDGN	15	0.853
STH	WorldView-2 CDGN	16	0.853
STH	WorldView-2 CDGN	20	0.853
STH	GeoEye-1 CDGN	3	0.849
STH	GeoEye-1 CDGN	4	0.848
STH	WorldView-2 CDGN	9	0.825
STH	WorldView-2 CDGN	1	0.824
STH	WorldView-2 CDGN	2	0.824
STH	WorldView-2 CDGN	4	0.824
STH	WorldView-2 CDGN	5	0.824
STH	WorldView-2 CDGN	7	0.824
STH	WorldView-2 CDGN	8	0.824
STH	WorldView-2 CDGN	10	0.824
STH	WorldView-2 CDGN	11	0.824
STH	WorldView-2 CDGN	12	0.824
STH	WorldView-2 CDGN	14	0.824
STH	WorldView-2 CDGN	17	0.824
STH	WorldView-2 CDGN	18	0.824
STH	WorldView-2 CDGN	19	0.807
STH	WorldView-2 CDGN	13	0.784
STH	WorldView-2 CDGN	21	0.710
STH	WorldView-2 CDGN	22	0.500

Table 4.12. GeoEye-1 and WorldView-2 model AUC values for hookworm in Campo Do Gado Novo (CDGN).

Parasite	Satellite/Area	Model Number	AUC
Hookworm	GeoEye-1 CDGN	1	0.922
Hookworm	GeoEye-1 CDGN	2	0.922
Hookworm	GeoEye-1 CDGN	5	0.922
Hookworm	WorldView-2 CDGN	3	0.894
Hookworm	WorldView-2 CDGN	6	0.894
Hookworm	WorldView-2 CDGN	15	0.894
Hookworm	WorldView-2 CDGN	16	0.894
Hookworm	WorldView-2 CDGN	20	0.894
Hookworm	WorldView-2 CDGN	1	0.854
Hookworm	WorldView-2 CDGN	2	0.854
Hookworm	WorldView-2 CDGN	4	0.854
Hookworm	WorldView-2 CDGN	5	0.854
Hookworm	WorldView-2 CDGN	7	0.854
Hookworm	WorldView-2 CDGN	8	0.854
Hookworm	WorldView-2 CDGN	10	0.854
Hookworm	WorldView-2 CDGN	11	0.854
Hookworm	WorldView-2 CDGN	12	0.854
Hookworm	WorldView-2 CDGN	14	0.854
Hookworm	WorldView-2 CDGN	17	0.854
Hookworm	WorldView-2 CDGN	18	0.854
Hookworm	GeoEye-1 CDGN	3	0.843
Hookworm	GeoEye-1 CDGN	4	0.843
Hookworm	WorldView-2 CDGN	9	0.839
Hookworm	WorldView-2 CDGN	13	0.820
Hookworm	WorldView-2 CDGN	19	0.808
Hookworm	WorldView-2 CDGN	21	0.686
Hookworm	WorldView-2 CDGN	22	0.641

Table 4.13. GeoEye-1 and WorldView-2 model AUC values for hookworm in Maria Quiteria-1.

Parasite	Model Number	Satellite/Area	AUC
Hookworm	1	GeoEye-1 Maria Quiteria-1	0.820
Hookworm	2	GeoEye-1 Maria Quiteria-1	0.820
Hookworm	3	GeoEye-1 Maria Quiteria-1	0.808
Hookworm	4	GeoEye-1 Maria Quiteria-1	0.808
Hookworm	6	WorldView-2 Maria Quiteria-1	0.704
Hookworm	4	WorldView-2 Maria Quiteria-1	0.703
Hookworm	5	WorldView-2 Maria Quiteria-1	0.703
Hookworm	13	WorldView-2 Maria Quiteria-1	0.703
Hookworm	1	WorldView-2 Maria Quiteria-1	0.657
Hookworm	2	WorldView-2 Maria Quiteria-1	0.657
Hookworm	3	WorldView-2 Maria Quiteria-1	0.657
Hookworm	8	WorldView-2 Maria Quiteria-1	0.657
Hookworm	9	WorldView-2 Maria Quiteria-1	0.657
Hookworm	11	WorldView-2 Maria Quiteria-1	0.657
Hookworm	16	WorldView-2 Maria Quiteria-1	0.657
Hookworm	22	WorldView-2 Maria Quiteria-1	0.657
Hookworm	12	WorldView-2 Maria Quiteria-1	0.626
Hookworm	14	WorldView-2 Maria Quiteria-1	0.626
Hookworm	15	WorldView-2 Maria Quiteria-1	0.626
Hookworm	21	WorldView-2 Maria Quiteria-1	0.626
Hookworm	7	WorldView-2 Maria Quiteria-1	0.500
Hookworm	10	WorldView-2 Maria Quiteria-1	0.500
Hookworm	17	WorldView-2 Maria Quiteria-1	0.500
Hookworm	18	WorldView-2 Maria Quiteria-1	0.500
Hookworm	19	WorldView-2 Maria Quiteria-1	0.500
Hookworm	20	WorldView-2 Maria Quiteria-1	0.500
Hookworm	5	GeoEye-1 Maria Quiteria-1	0.500

Table 4.14. GeoEye-1 model AUC values for hookworm in Maria Quiteria-2.

Parasite	Model Number	Satellite/Area	AUC
Hookworm	1	GeoEye-1 Maria Quiteria-2	0.818
Hookworm	2	GeoEye-1 Maria Quiteria-2	0.818
Hookworm	3	GeoEye-1 Maria Quiteria-2	0.765
Hookworm	4	GeoEye-1 Maria Quiteria-2	0.766
Hookworm	5	GeoEye-1 Maria Quiteria-2	0.500

For Maria Quiteria-2, 5 total ENMs were created to evaluate hookworm infections using the GE1 satellite. Hookworm infections were the only STH detected in this area, and so were the only STH evaluated. For hookworm ENMs, 11 presence points were used and the highest ENM AUCs were for GE1 Maria Quiteria-2 model 1 and GE1 Maria Quiteria-2 model 2 with an AUC=0.818 (Table 4.13.).

Overall, using AUC values, the best performing models in Queimadinha were WV2 model 9 (NDWI, NDVI), WV2 model 13 (WVWI, WVSI), and WV2 model 22 (WVSI) for STH, hookworm, *A. lumbricoides*, and *T. trichiura*. For CDGN the best performing model was GE1 model 5 (NDWI) for STH and GE1 model 1 (NDWI, NDVI), GE1 model 2 (SAVI, NDWI), and GE1 model 5 (NDWI) for hookworm. For Maria Quiteria-1 the best performing models were GE1 model 1 (NDVI, NDWI) and GE1 model 2 (SAVI, NDWI).

4.3.3. Comparison of WorldView-2 and GeoEye-1 Ecological Niche Models Using Sampled Sized-Corrected Akaike Information Criterion (AIC_c) Ranking

To determine the best performing VHR ENMs for each area, AIC_c values were calculated for models with AUCs>0.7. For Queimadinha STH, hookworm, *A. lumbricoides*, and *T. trichiura* ENMs were ranked. A total of 18 STH ENMs were evaluated using ENMTools and the best performing ENM was GE1 model 1, with an AIC_c=171.599248 (Table 4.15.). The top contributing variable for STH GE1 model 1 was the vegetation index NDVI, with 100% model contribution. For hookworm in Queimadinha, a total of 16 ENMs were evaluated and the best performing model evaluated was GE1 model 1, with an AIC_c=95.49679642 (Table 4.16.). The top contributing variable for hookworm GE1 model 1 was the vegetation index NDVI, with 100% model contribution. For the *A. lumbricoides* in Queimadinha a total of 23 ENMs were evaluated and the best performing model was WV2 model 13, with an AIC_c=116.1267087 (Table 4.17.). The top

contributing variable for *A. lumbricoides* WV2 model 13 was the soil index WVSI, with 100% model contribution. For *T. trichiura* in Queimadinha, a total of 3 ENMs were evaluated and the best performing models were WV2 model 9, 13, and 22, all with an AIC_c=80.73185793 (Table 4.18.). The top contributing variable for these 3 models was the soil index WVSI, with 100% model contribution.

Table 4.15 GeoEye-1 and WorldView-2 model AICc scores for STH in Queimadinha.

Parasite	Satellite/Area	Model Number	AICc score
STH	GeoEye-1 Queimadinha	1	171.599248
STH	GeoEye-1 Queimadinha	2	171.599405
STH	GeoEye-1 Queimadinha	3	171.599405
STH	GeoEye-1 Queimadinha	4	171.5994493
STH	WorldView-2 Queimadinha	16	172.6013948
STH	WorldView-2 Queimadinha	9	173.6137176
STH	WorldView-2 Queimadinha	13	173.6137176
STH	WorldView-2 Queimadinha	22	173.6137176
STH	WorldView-2 Queimadinha	1	174.9937974
STH	WorldView-2 Queimadinha	11	174.9940233
STH	WorldView-2 Queimadinha	4	174.9942464
STH	WorldView-2 Queimadinha	8	174.9954599
STH	WorldView-2 Queimadinha	2	175.0798347
STH	WorldView-2 Queimadinha	5	175.0922814
STH	WorldView-2 Queimadinha	3	176.1233988
STH	WorldView-2 Queimadinha	6	176.1239658
STH	WorldView-2 Queimadinha	10	176.418779
STH	WorldView-2 Queimadinha	7	176.4217086

Table 4.16. GeoEye-1 and WorldView-2 model AICc scores for hookworm in Queimadinha.

Parasite	Satellite/Area	Model Number	AICc score
Hookworm	GeoEye-1 Queimadinha	1	95.49679642
Hookworm	GeoEye-1 Queimadinha	3	95.49698966
Hookworm	GeoEye-1 Queimadinha	2	95.4983501
Hookworm	GeoEye-1 Queimadinha	4	95.4983501
Hookworm	WorldView-2 Queimadinha	13	97.68752853
Hookworm	WorldView-2 Queimadinha	22	97.68752853
Hookworm	WorldView-2 Queimadinha	9	97.68752854
Hookworm	WorldView-2 Queimadinha	2	103.732703
Hookworm	WorldView-2 Queimadinha	5	103.734128
Hookworm	WorldView-2 Queimadinha	11	103.7388379
Hookworm	WorldView-2 Queimadinha	1	103.7395299
Hookworm	WorldView-2 Queimadinha	4	103.7395299
Hookworm	WorldView-2 Queimadinha	8	103.7395299
Hookworm	WorldView-2 Queimadinha	3	104.3188018
Hookworm	WorldView-2 Queimadinha	6	104.3188018
Hookworm	WorldView-2 Queimadinha	16	104.3188018

Table 4.17. GeoEye-1 and WorldView-2 model AICc scores for *Ascaris lumbricoides* in Queimadinha.

Parasite	Satellite/Area	Model Number	AICc score
A. lumbricoides	WorldView-2 Queimadinha	13	116.1267087
A. lumbricoides	WorldView-2 Queimadinha	22	116.1267087
A. lumbricoides	WorldView-2 Queimadinha	9	116.1267088
A. lumbricoides	GeoEye-1 Queimadinha	4	116.1440935
A. lumbricoides	GeoEye-1 Queimadinha	3	116.1445552
A. lumbricoides	WorldView-2 Queimadinha	18	116.8117396
A. lumbricoides	WorldView-2 Queimadinha	17	116.8118664
A. lumbricoides	WorldView-2 Queimadinha	20	116.9524808
A. lumbricoides	WorldView-2 Queimadinha	8	119.7414595
A. lumbricoides	WorldView-2 Queimadinha	2	119.7721504
A. lumbricoides	WorldView-2 Queimadinha	1	119.7728859
A. lumbricoides	WorldView-2 Queimadinha	5	119.7791612
A. lumbricoides	WorldView-2 Queimadinha	4	119.7798969
A. lumbricoides	WorldView-2 Queimadinha	3	119.8830668
A. lumbricoides	WorldView-2 Queimadinha	6	119.8830668
A. lumbricoides	WorldView-2 Queimadinha	16	119.8830668
A. lumbricoides	WorldView-2 Queimadinha	10	120.4311507
A. lumbricoides	WorldView-2 Queimadinha	7	120.433053
A. lumbricoides	GeoEye-1 Queimadinha	2	121.1195121
A. lumbricoides	GeoEye-1 Queimadinha	1	121.12073
A. lumbricoides	WorldView-2 Queimadinha	14	121.136362
A. lumbricoides	WorldView-2 Queimadinha	12	121.1380393
A. lumbricoides	WorldView-2 Queimadinha	15	121.7657256

Table 4.18. GeoEye-1 and WorldView-2 model AICc scores for *Trichuris trichiura* in Queimadinha.

Parasite	Satellite/Area	Model Number	AICc score
T. trichiura	WorldView-2 Queimadinha	9	80.73185793
T. trichiura	WorldView-2 Queimadinha	22	80.73185793
T. trichiura	WorldView-2 Queimadinha	13	80.73185793

For CDGN, STH and hookworm ENM AIC_c scores were ranked. A total of 25 STH ENMs were evaluated using ENMTools and the best performing ENM was GE1 model 5, with an AIC_c=146.1031124 (Table 4.19.). The top contributing variable for STH GE1 model 5 was the water index NDWI, with 100% model contribution. For hookworm in CDGN a total of 18 STH ENMs were evaluated using ENMTools and the best performing ENMs were GE1 model 1 and 2, with an AIC_c=122.1542984 (Table 4.20.). The top contributing variables for STH GE1 model 1 and 2 were the vegetation indices NDVI and SAVI respectively, both with 95.4% model contribution.

For Maria Quiteria-1, hookworm ENM AIC_c scores were ranked. A total of 8 hookworm ENMs were evaluated using ENMTools and the best performing ENM was GE1 model 3, with an AIC_c=124.3804917 (Table 4.21.). The top contributing variable for STH GE1 model 5 was the water index NDWI, with 100% model contribution.

For Maria Quiteria-2, GE1 hookworm ENM AIC_c scores were ranked. A total of 4 hookworm ENMs were evaluated using ENMTools and the best performing ENM was GE1 model 1, with an AIC_c=325.1545496 (Table 4.22.). The top contributing variable for hookworm GE1 model 1 was the vegetation index NDVI, with 92% model contribution.

Table 4.19. GeoEye-1 and WorldView-2 model AICc scores for STH in Campo Do Gado Novo (CDGN).

Parasite	Satellite/Area	Model Number	AICc score
STH	GeoEye-1 CDGN	5	146.1031124
STH	GeoEye-1 CDGN	4	149.4813587
STH	GeoEye-1 CDGN	3	149.4841279
STH	WorldView-2 CDGN	3	149.806417
STH	WorldView-2 CDGN	15	149.806417
STH	WorldView-2 CDGN	6	149.806417
STH	WorldView-2 CDGN	16	149.806417
STH	GeoEye-1 CDGN	2	151.1240905
STH	GeoEye-1 CDGN	1	151.1241388
STH	WorldView-2 CDGN	18	151.2281031
STH	WorldView-2 CDGN	2	151.2285281
STH	WorldView-2 CDGN	10	151.2285281
STH	WorldView-2 CDGN	5	151.2285281
STH	WorldView-2 CDGN	11	151.2285281
STH	WorldView-2 CDGN	14	151.2285281
STH	WorldView-2 CDGN	17	151.2286524
STH	WorldView-2 CDGN	1	151.2291033
STH	WorldView-2 CDGN	7	151.2291033
STH	WorldView-2 CDGN	12	151.2291033
STH	WorldView-2 CDGN	4	151.2291033
STH	WorldView-2 CDGN	8	151.2291033
STH	WorldView-2 CDGN	19	152.8687057
STH	WorldView-2 CDGN	21	155.9304153
STH	WorldView-2 CDGN	9	156.809067
STH	WorldView-2 CDGN	13	160.3892669

Table 4.20. GeoEye-1 and WorldView-2 model AICc scores for hookworm in Campo Do Gado Novo (CDGN).

Parasite	Satellite/Area	Model Number	AICc score
Hookworm	GeoEye-1 CDGN	2	122.1542984
Hookworm	GeoEye-1 CDGN	1	122.1542984
Hookworm	GeoEye-1 CDGN	5	122.154494
Hookworm	WorldView-2 CDGN	20	123.3061296
Hookworm	WorldView-2 CDGN	3	123.3061303
Hookworm	WorldView-2 CDGN	6	123.3061303
Hookworm	WorldView-2 CDGN	15	123.3061303
Hookworm	WorldView-2 CDGN	16	123.3061303
Hookworm	WorldView-2 CDGN	17	125.440012
Hookworm	WorldView-2 CDGN	1	125.4401658
Hookworm	WorldView-2 CDGN	4	125.4401658
Hookworm	WorldView-2 CDGN	7	125.4401658
Hookworm	WorldView-2 CDGN	8	125.4401658
Hookworm	WorldView-2 CDGN	12	125.4401658
Hookworm	WorldView-2 CDGN	18	125.4402453
Hookworm	WorldView-2 CDGN	5	125.4403929
Hookworm	WorldView-2 CDGN	11	125.4403929
Hookworm	WorldView-2 CDGN	2	125.4403929
Hookworm	WorldView-2 CDGN	10	125.4403929
Hookworm	WorldView-2 CDGN	14	125.4403929
Hookworm	GeoEye-1 CDGN	4	125.7770501
Hookworm	GeoEye-1 CDGN	3	125.779444
Hookworm	WorldView-2 CDGN	19	128.321781
Hookworm	WorldView-2 CDGN	9	133.5951468
Hookworm	WorldView-2 CDGN	13	137.3981923

Table 4.21. GeoEye-1 and WorldView-2 model AICc scores for hookworm in Maria Quiteria-1.

Parasite	Satellite/Area	Model Number	AICc score
Hookworm	GeoEye-1 Maria Quiteria-1	3	124.3804917
Hookworm	GeoEye-1 Maria Quiteria-1	4	124.385104
Hookworm	GeoEye-1 Maria Quiteria-1	1	130.4681384
Hookworm	GeoEye-1 Maria Quiteria-1	2	130.4729781
Hookworm	WorldView-2 Maria Quiteria-1	4	136.241036
Hookworm	WorldView-2 Maria Quiteria-1	5	136.241036
Hookworm	WorldView-2 Maria Quiteria-1	13	136.241036
Hookworm	WorldView-2 Maria Quiteria-1	6	136.2754611

Table 4.22. GeoEye-1 model AICc scores for hookworm in Maria Quiteria-2.

Parasite	Satellite/Area	Sample Size	AICc score
Hookworm	GeoEye-1 Maria Quiteria-2	1	325.1545496
Hookworm	GeoEye-1 Maria Quiteria-2	2	325.1605952
Hookworm	GeoEye-1 Maria Quiteria-2	3	325.3360609
Hookworm	GeoEye-1 Maria Quiteria-2	4	325.3414653

4.3.4. Comparison of GeoEye-1 and Landsat 8 Ecological Niche Models Using Area Under the Curve (AUC) Values

Using the comparisons of ENM AIC_c scores for both VHR satellites evaluating STH across the Queimadinha, CDGN, and Maria Quiteria, it was determined that GE1 consistently produced the best performing ENMs. Therefore, our next step was to determine if GE1 ENMs provided improved model performance compared to the more widely used 30 m resolution satellite Landsat 8.

30 total ENMs using Landsat 8 were produced for the four study areas of Queimadinha, CDGN, Maria Quiteria-1, and Maria Quiteria-2 and model AUC values were compared with GE1 ENMs. In Queimadinha, the GE1 and Landsat 8 ENMs evaluating STH and *A. lumbricoides* were compared. Landsat 8 ENMs could not be produced for hookworm or *T. trichiura* due to each having only 2 presence points. For STH, Landsat 8 used 5 presence points compared to the 9 presence points utilized by GE1. The best performing ENMs were GE1 models 1 (Figure 4.4.), 2,

3, and 4 with AUCs=0.726 (Table 4.23.). The main contributing environmental variable for GE1 models 1-4 was the vegetation indices NDVI (models 1,3) and SAVI (models 2,4), with 100% contribution for all models. Landsat 8 models could not be made for hookworm, but the best performing model overall for hookworm was GE1 model 1 (Table 4.16, Figure 4.5.) with the vegetation index NDVI contributing 100% to the ENM. For *A. lumbricoides*, Landsat 8 used 4 presence points compared to the 6 presence points utilized by GE1. The best performing ENMs were GE1 models 1 (Figure 4.6.) and 2, with AUCs=0.716 (Table 4.24.). The main contributing environmental variable for GE1 models 1 and 2 were the vegetation indices NDVI and SAVI, respectively, both with 100% model contribution.

In CDGN, the GE1 and Landsat 8 ENMs evaluating STH and hookworm were compared. Landsat 8 ENMs could not be produced for *A. lumbricoides* or *T. trichiura* due to having only 1 presence point for each. For STH, Landsat 8 used 4 presence points compared to the 6 points used by GE1. The best performing ENM with the highest AUC value was GE1 model 5 (Figure 4.7.), with an AUC=0.921 (Table 4.25.). The main contributing variable in the GE1 model 5 was NDWI, with a 95.4% ENM contribution. For hookworm in CDGN, Landsat 8 used 3 presence points compared to the 5 points used by GE1. The best performing ENMs were GE1 models 1 (Figure 4.8.), 2, and 5, with an AUC=0.922 (Table 4.26.). The main contributing variable in all 3 ENMs was NDWI, with 95.4% contribution in GE1 models 1 and 2 and 100% contribution in model 5. In Maria Quiteria-1 and Maria Quiteria-2, only hookworm was detected during sampling. For Maria-Quiteria-1 4 presence points were used compared to 5 presence points used by GE1. The best performing ENMs were GE1 models 1 (Figure 4.9.) and 2, with AUCs=0.820 (Table 4.27.). The main contributing environmental variable for GE1 models 1 (Figure 4.10.) and 2 were the

vegetation indices NDVI and SAVI, respectively, both with 99.7% model contribution. For Maria Quiteria-2, Landsat 8 used 9 points compared to the 11 utilized by GE1. The best performing ENMs were GE1 models 1 and 2, with AUCs=0.818 (Table 4.28). The main contributing variable for GE1 models 1 and 2 were NDVI and SAVI, respectively, both with 92% model contribution.

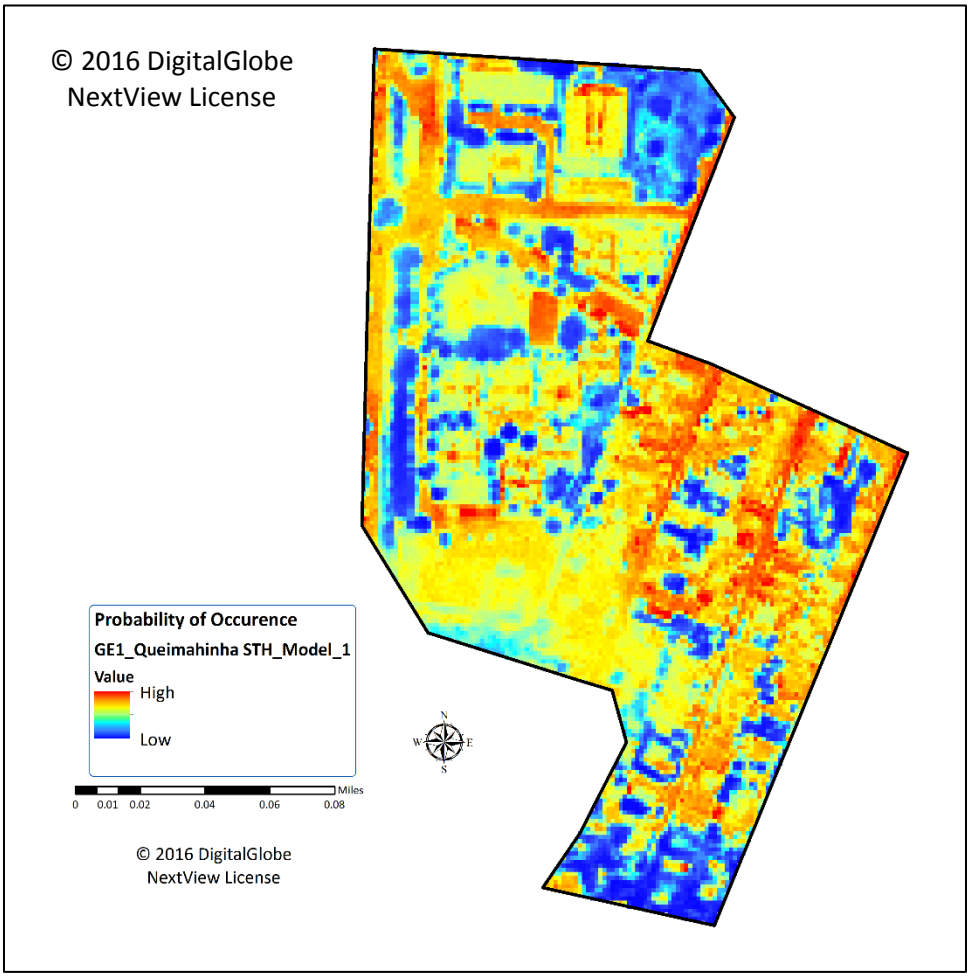

Figure 4.4. The GeoEye-1 (GE1) Maxent ecological niche model number 1, where NDVI was the main contributing variable, for soil-transmitted helminth (STH) occurrence probability in the Queimadinha census tract evaluated.

Table 4.23. GeoEye-1 and Landsat 8 model AUC values for STH in Queimadinha.

Parasite	Satellite/Area	Model Number	AUC
STH	GeoEye-1 Queimadinha	1	0.726
STH	GeoEye-1 Queimadinha	2	0.726
STH	GeoEye-1 Queimadinha	3	0.726
STH	GeoEye-1 Queimadinha	4	0.726
STH	Landsat 8 Queimadinha	1	0.629
STH	Landsat 8 Queimadinha	2	0.629
STH	Landsat 8 Queimadinha	3	0.629
STH	Landsat 8 Queimadinha	4	0.629
STH	GeoEye-1 Queimadinha	5	0.557
STH	Landsat 8 Queimadinha	5	0.500

Table 4.24. GeoEye-1 and Landsat 8 model AUC values for *Ascaris lumbricoides* in Queimadinha.

Parasite	Satellite/Area	Model Number	AUC
A. lumbricoides	GeoEye-1 Queimadinha	1	0.716
A. lumbricoides	GeoEye-1 Queimadinha	2	0.716
A. lumbricoides	GeoEye-1 Queimadinha	3	0.715
A. lumbricoides	GeoEye-1 Queimadinha	4	0.715
A. lumbricoides	Landsat 8 Queimadinha	1	0.647
A. lumbricoides	Landsat 8 Queimadinha	2	0.647
A. lumbricoides	Landsat 8 Queimadinha	5	0.647
A. lumbricoides	GeoEye-1 Queimadinha	5	0.500
A. lumbricoides	Landsat 8 Queimadinha	3	0.500
A. lumbricoides	Landsat 8 Queimadinha	4	0.500

Table 4.25. GeoEye-1 and Landsat 8 model AUC values for STH in in Campo Do Gado Novo (CDGN).

Parasite	Satellite/Area	Model Number	AUC
STH	GeoEye-1 CDGN	5	0.921
STH	GeoEye-1 CDGN	1	0.919
STH	GeoEye-1 CDGN	2	0.919
STH	GeoEye-1 CDGN	3	0.849
STH	GeoEye-1 CDGN	4	0.848
STH	Landsat 8 CDGN	1	0.796
STH	Landsat 8 CDGN	2	0.796
STH	Landsat 8 CDGN	3	0.796
STH	Landsat 8 CDGN	4	0.796
STH	Landsat 8 CDGN	5	0.698

Table 4.26. GeoEye-1 and Landsat 8 model AUC values for hookworm in Campo Do Gado Novo (CDGN).

Parasite	Satellite/Area	Model Number	AUC
Hookworm	GeoEye-1 CDGN	1	0.922
Hookworm	GeoEye-1 CDGN	2	0.922
Hookworm	GeoEye-1 CDGN	5	0.922
Hookworm	GeoEye-1 CDGN	3	0.843
Hookworm	GeoEye-1 CDGN	4	0.843
Hookworm	Landsat 8 CDGN	1	0.811
Hookworm	Landsat 8 CDGN	2	0.811
Hookworm	Landsat 8 CDGN	3	0.811
Hookworm	Landsat 8 CDGN	4	0.811
Hookworm	Landsat 8 CDGN	5	0.704

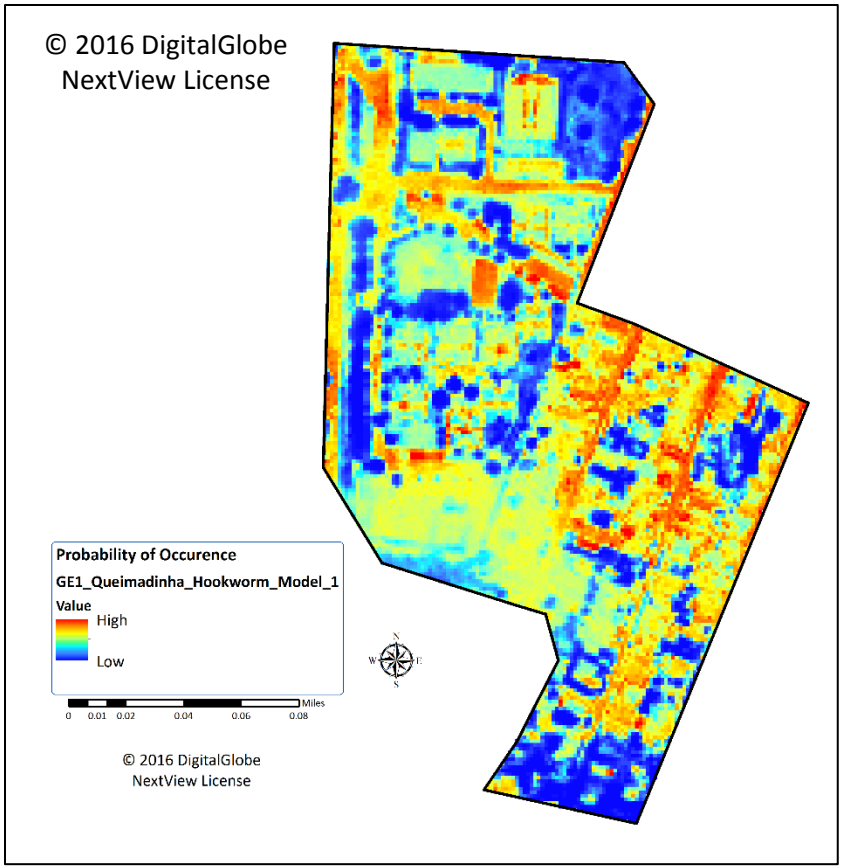

Figure 4.5. The GeoEye-1 (GE1) Maxent ecological niche model number 1, where NDVI was the main contributing variable, for hookworm occurrence probability in the Queimadinha census tract evaluated.

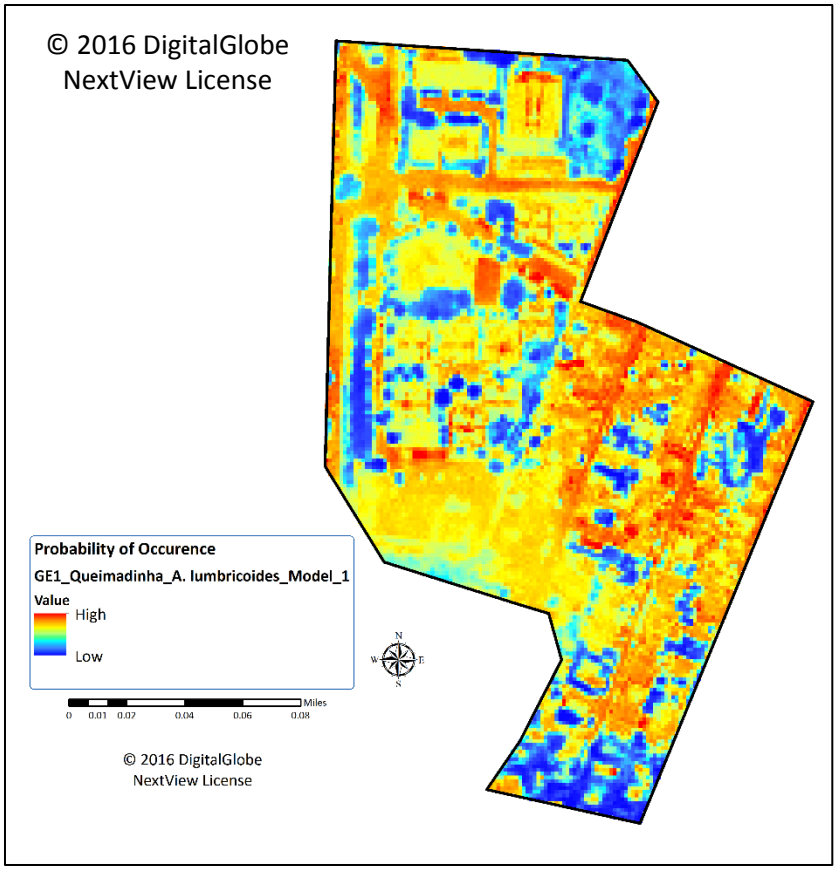

Figure 4.6. The GeoEye-1 (GE1) Maxent ecological niche model number 1, where NDVI was the main contributing variable, for *Ascaris lumbricoide*s occurrence probability in the Queimadinha census tract evaluated.

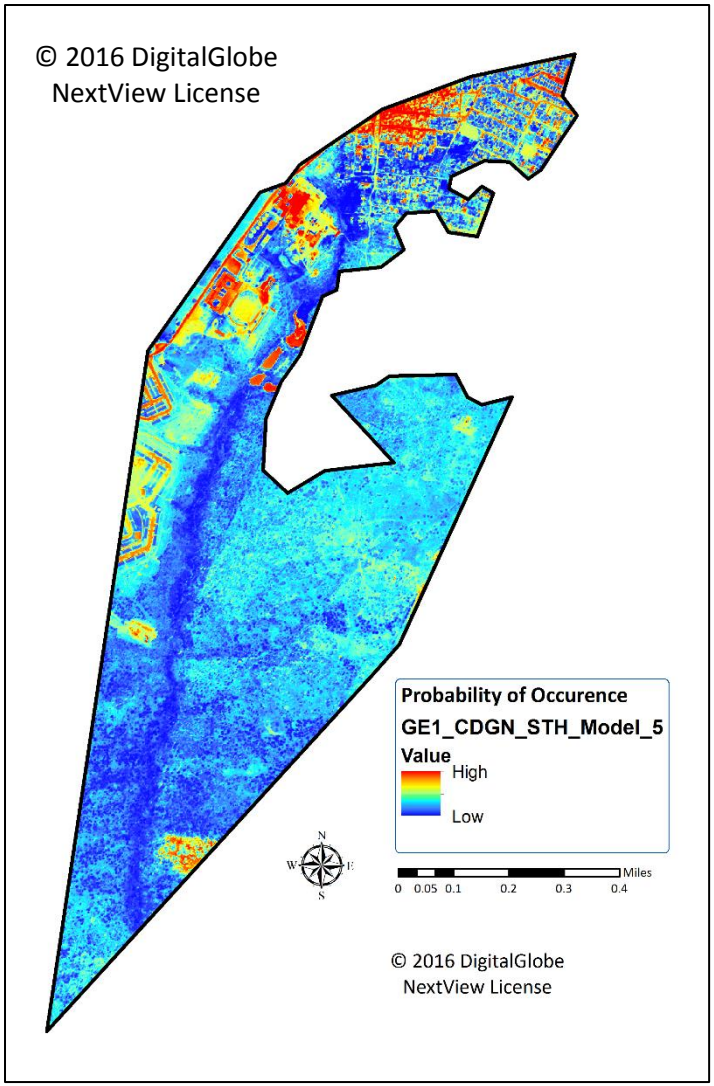

Figure 4.7. The GeoEye-1 (GE1) Maxent ecological niche model number 5, where NDWI was the main contributing variable, for soil-transmitted helminth (STH) occurrence probability in the Campo Do Gado Novo C(DGN).

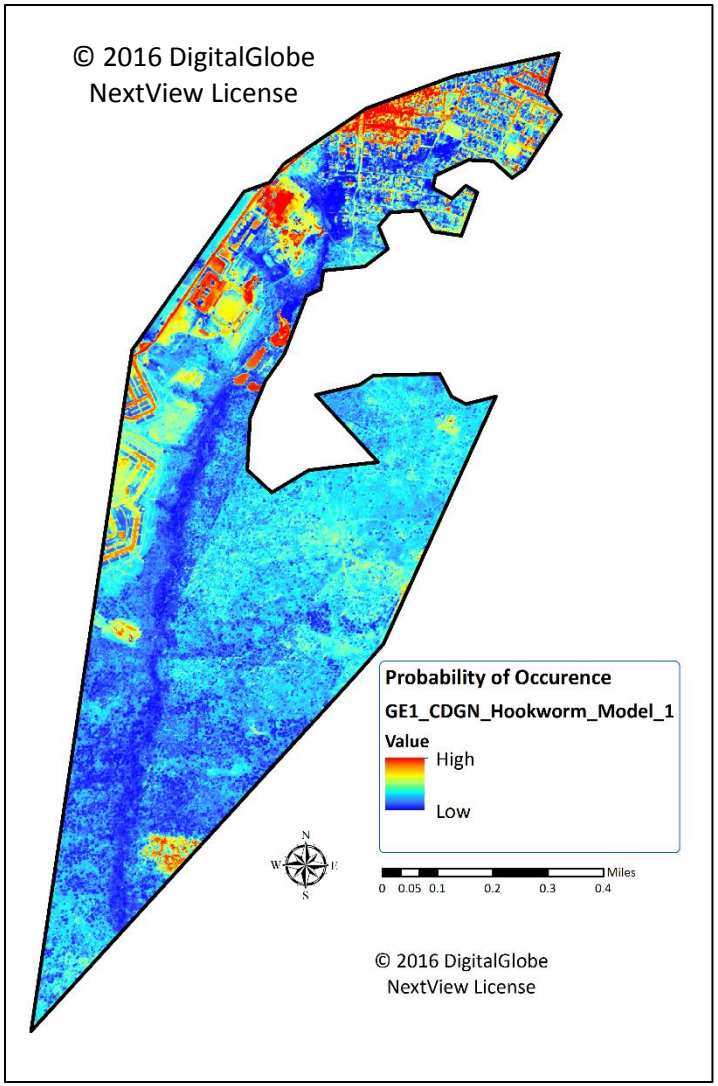

Figure 4.8. The GeoEye-1 (GE1) Maxent ecological niche model number 1, where NDWI was the main contributing variable, for hookworm occurrence probability in the Campo Do Gado Novo (CDGN).

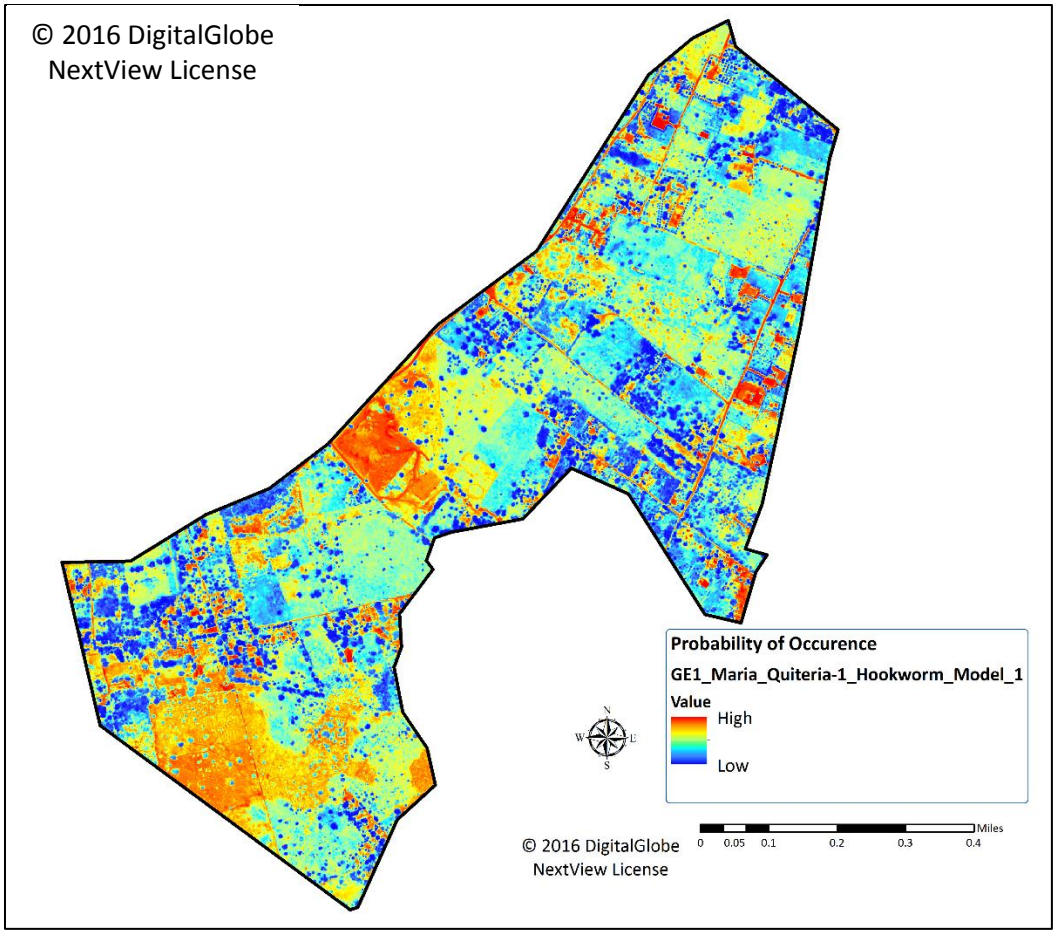

Figure 4.9. The GeoEye-1 (GE1) Maxent ecological niche model number 1, where NDVI was the main contributing variable, for hookworm occurrence probability in Maria Quiteria-1.

Table 4.27. GeoEye-1 and Landsat 8 model AUC values for hookworm in Maria Quiteria-1.

Parasite	Satellite/Area	Model Number	AUC
Hookworm	GeoEye-1 Maria Quiteria-1	1	0.820
Hookworm	GeoEye-1 Maria Quiteria-1	2	0.820
Hookworm	GeoEye-1 Maria Quiteria-1	3	0.808
Hookworm	GeoEye-1 Maria Quiteria-1	4	0.808
Hookworm	Landsat 8 Maria-Quiteria-1	1	0.765
Hookworm	Landsat 8 Maria-Quiteria-1	2	0.765
Hookworm	Landsat 8 Maria-Quiteria-1	5	0.650
Hookworm	GeoEye-1 Maria Quiteria-1	5	0.500
Hookworm	Landsat 8 Maria-Quiteria-1	3	0.500
Hookworm	Landsat 8 Maria-Quiteria-1	4	0.500

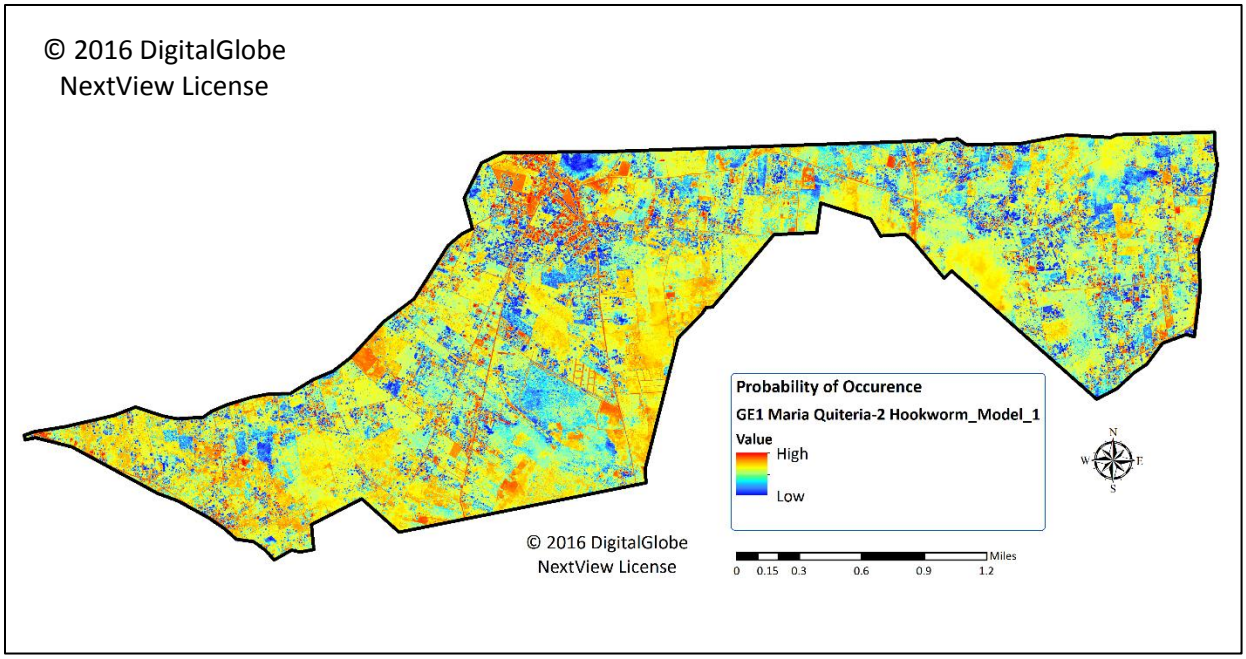

Figure 4.10. The GeoEye-1 (GE1) Maxent ecological niche model number 1, where NDVI was the main contributing variable, for hookworm occurrence probability in Maria Quiteria

Table 4.28. GeoEye-1 and Landsat 8 model AUC values for hookworm in Maria Quiteria-2.-2.

Parasite	Satellite/Area	Model Number	AUC
Hookworm	GeoEye-1 Maria Quiteria-2	1	0.818
Hookworm	GeoEye-1 Maria Quiteria-2	2	0.818
Hookworm	GeoEye-1 Maria Quiteria-2	4	0.766
Hookworm	GeoEye-1 Maria Quiteria-2	3	0.765
Hookworm	Landsat 8 Maria-Quiteria-2	1	0.700
Hookworm	Landsat 8 Maria-Quiteria-2	2	0.700
Hookworm	Landsat 8 Maria-Quiteria-2	5	0.700
Hookworm	GeoEye-1 Maria Quiteria-2	5	0.500
Hookworm	Landsat 8 Maria-Quiteria-2	3	0.500
Hookworm	Landsat 8 Maria-Quiteria-2	4	0.500

4.4. Discussion

This study in Feira de Santana demonstrates the utility of VHR satellite imagery in modeling STH ecological distribution and determining positively associated environmental variables. Comparing the GE1 and WV2 VHR satellites using AIC_c scores, we established that GE1 consistently produced superior performing ENMs for STH and hookworm infections across the three study communities compared to WV2. GE1 produced the best performing models for STH

and hookworm in Queimadinha, STH and hookworm in CDGN, and hookworm in Maria Quiteria-1. For *A. lumbricoides* in Queimadinha the WV2 ENMs performed nominally better than the GE1 ENMs (WV2 116.1267087 vs. 116.1440935 AIC_c). For *T. trichiura* in Queimadinha, only the WV2 produced informative (AUC>0.7) ENMs (Swets 1998; Baldwin 2009). However, these Queimadinha *T. trichiura* ENM results for should be viewed with caution, as only 4 occurrence points were available for modelling.

Comparing GE1 ENMS with those produced by Landsat 8 for Queimadinha, CDGN, Maria Quiteria-1, and Maria Quiteria-2, we demonstrated that GE1 produces superior performing STH ENMs. Many Landsat 8 ENMs were non-informative models, as they failed to produce ENMs with AUC values >0.7. The coarser Landsat 8 resolution also does not allow for the offsetting of household coordinates to more accurately account for multiple infections at a single location, which is an added benefit of using VHR satellite imagery. This limited our ability to compare the VHR and HR ENMs using AIC_c scores, as comparisons require an equivalent number of presence points, and contributed to the poor performance of the Landsat 8 ENMs. In fact, even separate coordinates that fall too close together within a 30 m pixel may be evaluated as a single spatial unit using HR imagery (Pullan et al. 2008), furthering decreasing the usable data. VHR satellites allow for these results confirm our hypothesis that a VHR satellite (GE1) more accurately models STH ecological distribution. The ability to model at this refined scale increases precision in risk assessment and subsequently, to develop a targeted treatment program within a given community. Ultimately, these benefits are driven by a better understanding of which environmental factors are important to STH distribution. This ability is required if a successful surveillance and response system for STH elimination is to be established in Feira de Santana.

The 30 m pixel resolution of Landsat 8 is too coarse to provide accurate distribution within a given community and does not allow the Feira de Santana MOH to selectively target areas within a community.

The successful use of VHR imagery to model at the household and its surrounding habitat allowed for the evaluation of major environmental variables associated with STH infections. In this study, we found that the major contribution variables of the ENM differed depending on whether the community was urban (where vegetation and soil were the main contributors), peri-urban (where water was the main contributor), or rural (where vegetation was the main contributor). This supports our original hypothesis that evaluating STH distribution on a scale of <5 m, which enables accurate modeling of households and their corresponding habitats, would reveal differences in environmental importance between the urban Queimadinha community, the peri-urban CDGN community, and the rural Maria Quiteria community.

In the urban community of Queimadinha, vegetation was revealed to be the most important variable for GE1 STH, *A. lumbricoides*, and hookworm ENMs. STH require soil moisture for development, and vegetation provides an indirect measurement of soil moisture and provides shade from intense direct sunlight, which can lead to desiccation (Saathoff, Olsen, Sharp, et al. 2005; Saathoff, Olsen, Kvalsvig, et al. 2005). This is particularly true for hookworm, which requires moisture to survive in its free-living stage and is susceptible to ultraviolet irradiation from the sun (Saathoff, Olsen, Sharp, et al. 2005). Our results are similar to previous studies demonstrating the positive association between vegetation and *A. lumbricoides* and hookworm infection (Saathoff, Olsen, Sharp, et al. 2005; Saathoff, Olsen, Kvalsvig, et al. 2005; Riess et al. 2013; Wardell et al. 2017; Midzi et al. 2018). It should be noted that the overall best performing ENMs

for *A. lumbricoides* in Queimadinha were produced by WV2, with soil as the main contributing variable. Soil also was the main contributing variable for the best *T. trichiura* WV2 ENMs for Queimadinha. The importance of soil may stem WV2 detecting the scarce amounts of bare soil found in urban environments where *A. lumbricoides* and *T. trichiura* eggs are developing, or possibly detecting sandy soils, which are favorable for *A. lumbricoides* development (Beaver 1952). The urban buildings may already provide the needed shade for STH development, and therefore scarce urban vegetation serves as a poor predictor of suitability. Conversely, the reason soil has no model importance in the peri-urban and rural communities may stem from the fact that bare soil is in these communities is unprotected from UV radiation, and vegetation is needed to provide the shade and moisture needed for STH development.

In the peri-urban community of CDGN, the water index was the most importance variable for the GE1 STH and hookworm ENMs. This community is characterized by the development of seasonal water bodies and proximity to these most likely provide the required soil moisture for STH development (Mudenda et al. 2012).

In Maria Quiteria-1 and Maria Quiteria-2, vegetation was the most important variable for GE1 hookworm ENMs. This mirrors the results for Queimadinha. The rural area is comprised mostly of semi-arid caatinga shrubs and farmland, and the vegetation growth found in this environment would provide moist, direct-sunlight protected, areas for hookworm development.

4.5. Conclusion

This study indicates that VHR satellites GE1 and WV2 can effectively and reliably be used to model STH distribution on a very high-resolution scale of <5 m to effectively evaluate the household and its surrounding habitat, whereas the use of the coarser resolution satellite

Landsat 8 is unable to model at this scale as effectively. The ability to effectively model at this scale allowed us to detect differences in which environmental variables were most important to STH distribution between the three communities. This study also demonstrates that the VHR GE1 satellite images, provides superior STH and hookworm distribution models and comparable *A. lumbricoides* distribution models compared to the more expensive WV2 satellite images and provides the modeling performance needed for a surveillance and response system in Feira de Santana. Our recommendation for Feira de Santana is to use the GE1 satellite as it produced superior performing ENMs for STH and hookworm, and comparable performing models for *A. lumbricoides*. GE1 has the additional benefit of being cheaper per sq. km than WV2 and has archived data that spans farther back than WV2 (LAND INFO Worldwide Mapping LLC 2018). While WV2 modeled *T. trichiura* better, the cost-benefit of using both GE1 and WV2 satellites is minimal, particularly in Feira de Santana, where the predominant STH infection is hookworm. To effectively administer a surveillance and response system geared at STH elimination in Feira de Santana, the ability to accurately identify and treat areas of high risk is needed. This study provides the framework, in conjunction with the Feira MOH, to begin implementing active surveillance and treatment in these 3 communities.

4.6. References

Akaike H. 1974. A New Look at the Statistical Model Identification. IEEE Trans. Automat. Contr. 19:716–723. doi:10.1109/TAC.1974.1100705.

Albonico M, Smith PG, Ercole E, Hall A, Chwaya HM, Alawi KS, Savioli L. 1995. Rate of reinfection with intestinal nematodes after treatment of children with mebendazole or albendazole in a highly endemic area.

Almeida PHA de, Santana PCS, Silva AV da. 2012. Prevalência de protozoários e helmintos entéricos em residentes de são cristóvão, feira de santana, bahia, brasil. Arq. Ciênc. Saúde UNIPAR 16:61–66.

Baldwin RA. 2009. Use of maximum entropy modeling in wildlife research. Entropy 11:854–866. doi:10.3390/e11040854.

Barbosa VS, Araújo KC, Leal Neto OB, Barbosa CS. 2012. Spatial distribution of schistosomiasis and geohelminthiasis cases in the rural areas of Pernambuco, Brazil. Rev. Soc. Bras. Med. Trop. 45:633–638. doi:10.1590/S0037-86822012000500017.

Beaver PC. 1952. Observations on the epidemiology of ascariasis in a region of high hookworm endemicity. J. Parasitol. 38:445–53.

Bergquist R, Yang GJ, Knopp S, Utzinger J, Tanner M. 2015. Surveillance and response: Tools and approaches for the elimination stage of neglected tropical diseases. Acta Trop. 141:229–234. doi:10.1016/j.actatropica.2014.09.017.

Bethony J, Brooker S, Albonico M, Geiger SM, Loukas A, Diemert D, Hotez PJ. 2006. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. Lancet 367:1521–1532. doi:10.1016/S0140-6736(06)68653-4.

Brooker S, Clements ACA, Bundy DAP. 2006. Global Epidemiology, Ecology and Control of Soil-Transmitted Helminth Infections. Adv. Parasitol. 62:221–261. doi:10.1016/S0065-308X(05)62007-6.

Chammartin F, Guimarães LH, Scholte RGC, Bavia ME, Utzinger J, Vounatsou P. 2014. Spatio-temporal distribution of soil-transmitted helminth infections in Brazil. Parasites and Vectors 7:440. doi:10.1186/1756-3305-7-440.

Chammartin F, Scholte RGC, Guimarães LH, Tanner M, Utzinger J, Vounatsou P. 2013. Soil-transmitted helminth infection in South America: a systematic review and geostatistical meta-analysis. Lancet. Infect. Dis. 13:507–18. doi:10.1016/S1473-3099(13)70071-9.

Deng Y, Wu C, Li M, Chen R. 2015. RNDSI: A ratio normalized difference soil index for remote sensing of urban/suburban environments. Int. J. Appl. Earth Obs. Geoinf. 39:40–48. doi:10.1016/J.JAG.2015.02.010.

DigitalGlobe Inc. 2014. Data Sheet GeoEye-1.

DigitalGlobe Inc. 2016. Data Sheet WorldView-2.

Diniz AF, Galvani E. 2014. ESTUDO DA PRECIPITAÇÃO PLUVIOMÉTRICA RELACIONADA COM A PRODUTIVIDADE DO MILHO NO MUNICÍPIO DE FEIRA DE SANTANA. Vitoria.

Dormann CF, Elith J, Bacher S, Buchmann C, Carl G, Carré G, Marquéz JRG, Gruber B, Lafourcade B, Leitão PJ, et al. 2013. Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. Ecography (Cop.). 36:27–46. doi:10.1111/j.1600-0587.2012.07348.x.

Elith J, Phillips SJ, Hastie T, Dudík M, Chee YE, Yates CJ. 2011. A statistical explanation of MaxEnt for ecologists. Divers. Distrib. 17:43–57. doi:10.1111/j.1472-4642.2010.00725.x.

Faria CP, Zanini GM, Dias GS, da Silva S, de Freitas MB, Almendra R, Santana P, Sousa M do C. 2017. Geospatial distribution of intestinal parasitic infections in Rio de Janeiro (Brazil) and its association with social determinants. Raso G, editor. PLoS Negl. Trop. Dis. 11:e0005445. doi:10.1371/journal.pntd.0005445.

Fithian W, Hastie T. 2013. Finite-sample equivalence in statistical models for presence-only data. Ann. Appl. Stat. 7:1917–1939. doi:10.1214/13-AOAS667.

Glenn EP, Huete AR, Nagler PL, Nelson SG. 2008. Relationship between remotely-sensed vegetation indices, canopy attributes and plant physiological processes: What vegetation indices can and cannot tell us about the landscape. Sensors 8:2136–2160. doi:10.3390/s8042136.

Huete A. 1988. A soil-adjusted vegetation index (SAVI). Remote Sens. Environ. 25:295–309. doi:10.1016/0034-4257(88)90106-X.

IBGE-Instituto Brasileiro de Geografia e Estatística. 2017. Brasil/Bahia/Feira de Santana. IBGE. [accessed 2019 Feb 12]. https://cidades.ibge.gov.br/brasil/ba/feira-de-santana/panorama.

IBGE. IBGE | Portal do IBGE. [accessed 2019 Mar 12]. https://www.ibge.gov.br/.

Inampudi RB. 1998. Image mosaicing. In: IGARSS '98. Sensing and Managing the Environment. 1998 IEEE International Geoscience and Remote Sensing. Symposium Proceedings. (Cat. No.98CH36174). IEEE. p. 2363–2365 vol.5.

Jiménez-Valverde A. 2012. Insights into the area under the receiver operating characteristic curve (AUC) as a discrimination measure in species distribution modelling. Glob. Ecol. Biogeogr. 21:498–507. doi:10.1111/j.1466-8238.2011.00683.x.

Jourdan PM, Lamberton PHL, Fenwick A, Addiss DG. 2018. Soil-transmitted helminth infections. Lancet 391:252–265. doi:10.1016/S0140-6736(17)31930-X.

LAND INFO Worldwide Mapping LLC. 2018. Satellite Imagery Pricing January, 2018. Littleton.

Li W, Du Z, Ling F, Zhou D, Wang H, Gui Y, Sun B, Zhang X. 2013. A Comparison of Land Surface Water Mapping Using the Normalized Difference Water Index from TM, ETM+ and ALI. Remote Sens. 5:5530–5549. doi:10.3390/rs5115530.

Lobo JM, Jiménez-valverde A, Real R. 2008. AUC: A misleading measure of the performance of predictive distribution models. Glob. Ecol. Biogeogr. 17:145–151. doi:10.1111/j.1466-8238.2007.00358.x.

Mabaso MLH, Appleton CC, Hughes JC, Gouws E. 2003. The effect of soil type and climate on hookworm (Necator americanus) distribution in KwaZulu-Natal, South Africa. Trop. Med. Int. Heal. 8:722–727. doi:10.1046/j.1365-3156.2003.01086.x.

Martins-Melo FR, Ramos AN, Alencar CH, Lima MS, Heukelbach J. 2017. Epidemiology of soil-

transmitted helminthiases-related mortality in Brazil. Parasitology 144:669–679. doi:10.1017/S0031182016002341.

Masek JG, Taylor MP, Rocchio L. 2019. Landsat 8 Overview « Landsat Science. [accessed 2019 Mar 12]. https://landsat.gsfc.nasa.gov/landsat-8/landsat-8-overview/.

McFeeters SK. 1996. The use of the Normalized Difference Water Index (NDWI) in the delineation of open water features. Int. J. Remote Sens. 17:1425–1432. doi:10.1080/01431169608948714.

Midzi N, Kavhu B, Manangazira P, Phiri I, Mutambu SL, Tshuma C, Chimbari MJ, Munyati S, Midzi SM, Charimari L, et al. 2018. Inclusion of edaphic predictors for enhancement of models to determine distribution of soil-transmitted helminths: The case of Zimbabwe. Parasites and Vectors 11:47. doi:10.1186/s13071-017-2586-6.

Ministério da Agricultura Pecuária e Abastecimento. INMET - Instituto Nacional de Meteorologia. [accessed 2019 Mar 11]. http://www.inmet.gov.br/portal/index.php?r=home2/index.

Monteiro KJL, Reis ERC Dos, Nunes BC, Jaeger LH, Calegar DA, Santos JP Dos, Maia A de O, Xavier SC das C, Bóia MN, Carvalho-Costa FA, et al. 2018. Focal persistence of soil-transmitted helminthiases in impoverished areas in the State of Piaui, Northeastern Brazil. Rev. Inst. Med. Trop. Sao Paulo 60:e24. doi:10.1590/s1678-9946201860024.

Moraes D. Invivo ::: Environment ::: The Caatinga biome. [accessed 2018 Oct 25]. http://www.invivo.fiocruz.br/cgi/cgilua.exe/sys/start.htm?UserActiveTemplate=espanol&infoid=1320&sid=102.

Mudenda NB, Malone JB, Kearney MT, Mischler PD, Del P, Nieto M, McCarroll JC, Vounatsou P. 2012. Modelling the ecological niche of hookworm in brazil based on climate. Geospat. Health 6.

National Council of the Atlantic Forest Biosphere Reserve. 2004. Reserva da Biosfera da Caatinga. [accessed 2019 Feb 14]. http://www.rbma.org.br/mab/unesco_03_rb_caatinga.asp.

NGA. 2017. NextView License Information Paper. Springfield.

Peterson AT, Papeş M, Soberón J. 2008. Rethinking receiver operating characteristic analysis applications in ecological niche modeling. Ecol. Modell. 213:63–72. doi:10.1016/j.ecolmodel.2007.11.008.

Phillips S. 2008. A Brief Tutorial on Maxent. AT&T Res. 3:1–38. doi:10.4016/33172.01.

Phillips SJ, Anderson RP, Dudík M, Schapire RE, Blair ME. 2017. Opening the black box: an open-source release of Maxent. Ecography (Cop.). 40:887–893. doi:10.1111/ecog.03049.

Phillips SJ, Anderson RP, Schapire RE. 2006. Maximum entropy modeling of species geographic distributions. Ecol. Modell. 190:231–259. doi:10.1016/j.ecolmodel.2005.03.026.

Phillips SJ, Dudík M, Schapire RE. Maxent software for modeling species niches and distributions (Version 3.4.1).

Phillips SJ, Dudík M, Schapire RE, Avenue P, Park F, Dudík M, Schapire RE. 2004. A maximum entropy approach to species distribution modeling. Twenty-first Int. Conf. Mach. Learn. - ICML '04:83. doi:10.1145/1015330.1015412.

Pullan RL, Bethony JM, Geiger SM, Cundill B, Correa-Oliveira R, Quinnell RJ, Brooker S. 2008. Human helminth co-infection: Analysis of spatial patterns and risk factors in a Brazilian community. PLoS Negl. Trop. Dis. 2. doi:10.1371/journal.pntd.0000352.

Pullan RL, Smith JL, Jasrasaria R, Brooker SJ. 2014. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. Parasit. Vectors 7:37. doi:10.1186/1756-3305-7-37.

Riess H, Clowes P, Kroidl I, Kowuor DO, Nsojo A, Mangu C, Schüle SA, Mansmann U, Geldmacher C, Mhina S, et al. 2013. Hookworm Infection and Environmental Factors in Mbeya Region, Tanzania: A Cross-Sectional, Population-Based Study. Jex AR, editor. PLoS Negl. Trop. Dis. 7:e2408. doi:10.1371/journal.pntd.0002408.

Saathoff E, Olsen A, Kvalsvig JD, Appleton CC, Sharp B, Kleinschmidt I. 2005. Ecological covariates of Ascaris lumbricoides infection in schoolchildren from rural KwaZulu-Natal, South Africa. Trop. Med. Int. Heal. 10:412–422. doi:10.1111/j.1365-3156.2005.01406.x.

Saathoff E, Olsen A, Sharp B, Kvalsvig JD, Appleton CC. 2005. Ecologic Covariates of Hookworm Infection and Reinfection in Rural Kwazulu-Natal/South Africa: a Geographic Information System-Based Study.

Scholte RGC, Freitas CC, Dutra L V., Guimaraes RJPS, Drummond SC, Oliveira G, Carvalho OS. 2012. Utilizing environmental, socioeconomic data and GIS techniques to estimate the risk for ascariasis and trichuriasis in Minas Gerais, Brazil. Acta Trop. 121:112–117. doi:10.1016/j.actatropica.2011.10.011.

Schule SA, Clowes P, Kroidl I, Kowuor DO, Nsojo A, Mangu C, Riess H, Geldmacher C, Laubender RP, Mhina S, et al. 2014. Ascaris lumbricoides Infection and Its Relation to Environmental Factors in the Mbeya Region of Tanzania, a Cross-Sectional, Population-Based Study. Keating JA, editor. PLoS One 9:e92032. doi:10.1371/journal.pone.0092032.

Sedano F, Kempeneers P, Strobl P, Kucera J, Vogt P, Seebach L, San-Miguel-Ayanz J. 2011. A cloud mask methodology for high resolution remote sensing data combining information from high and medium resolution optical sensors. ISPRS J. Photogramm. Remote Sens. 66:588–596. doi:10.1016/j.isprsjprs.2011.03.005.

Sun L, Mi X, Wei J, Wang J, Tian X, Yu H, Gan P. 2017. A cloud detection algorithm-generating method for remote sensing data at visible to short-wave infrared wavelengths. ISPRS J. Photogramm. Remote Sens. 124:70–88. doi:10.1016/j.isprsjprs.2016.12.005.

Swets JA. 1998. Measuring the Accuracy of Diagnostic Systems. Science (80-.). 240:1285–93.

Tambo E, Ai L, Zhou X, Chen J-H, Hu W, Bergquist R, Guo J-G, Utzinger J, Tanner M, Zhou X-N. 2014. Surveillance-response systems: the key to elimination of tropical diseases. Infect. Dis. poverty 3:17. doi:10.1186/2049-9957-3-17.

Tucker CJ. 1979. Red and photographic infrared linear combinations for monitoring vegetation. Remote Sens. Environ. 8:127–150. doi:10.1016/0034-4257(79)90013-0.

Wardell R, Clements ACA, Lal A, Summers D, Llewellyn S, Campbell SJ, McCarthy J, Gray DJ, V. Nery S. 2017. An environmental assessment and risk map of Ascaris lumbricoides and Necator americanus distributions in Manufahi District, Timor-Leste. de Silva N, editor. PLoS Negl. Trop. Dis. 11:e0005565. doi:10.1371/journal.pntd.0005565.

Warren DL, Glor RE, Turelli M. 2010. ENMTools: A toolbox for comparative studies of environmental niche models. Ecography (Cop.). 33:607–611. doi:10.1111/j.1600-0587.2009.06142.x.

Warren DL, Seifert SN. 2011. Ecological niche modeling in Maxent: The importance of model complexity and the performance of model selection criteria. Ecol. Appl. 21:335–342. doi:10.1890/10-1171.1.

WHO. 2012. Soil-transmitted helminthiases : eliminating as public health problem soil-transmitted helminthiases in children : progress report 2001-2010 and strategic plan 2011-2020. Geneva: World Health Organization.

Wolf AF. 2012. Using WorldView-2 Vis-NIR multispectral imagery to support land mapping and feature extraction using normalized difference index ratios. In: Shen SS, Lewis PE, editors. Vol. 8390. International Society for Optics and Photonics. p. 83900N–83900N–8.

Wolf MA. 2010. Using WorldView 2 Vis-NIR MSI Imagery to Support Land Mapping and Feature Extraction Using Normalized Difference Index Ratios.

Xue J, Su B. 2017. Significant Remote Sensing Vegetation Indices: A Review of Developments and Applications. J. Sensors 2017:1–17. doi:10.1155/2017/1353691.

Yackulic CB, Chandler R, Zipkin EF, Royle JA, Nichols JD, Campbell Grant EH, Veran S. 2013. Presence-only modelling using MAXENT: When can we trust the inferences? Methods Ecol. Evol. 4:236–243. doi:10.1111/2041-210x.12004.

Chapter 5: Molecular Confirmation of *Ascaris suum*: Further Investigation into the Zoonotic Origin of Infection in an 8-Year-Old Boy with Loeffler Syndrome[1]

5.1. Introduction

Ascaris lumbricoides and *Ascaris suum* are gastrointestinal nematodes that occur worldwide in humans and swine, respectively. *Ascaris* are transmitted via a direct fecal-oral cycle and are closely linked with poverty and poor sanitation. Often asymptomatic, *Ascaris* can cause intestinal pain, malnutrition, and impaired cognitive and physical development (Bethony et al. 2006). In the lung migratory phase, larval *Ascaris* may cause severe pulmonary eosinophilic pneumonitis known as Loeffler syndrome. While *A. lumbricoides* is considered a human pathogen and *A. suum* a swine pathogen, *A. suum* has been reported in humans, *A. lumbricoides* has been reported in swine, and cross-hybridization between the two has been demonstrated as reviewed by Nejsum et al. 2012. While most prevalent in developing countries, human ascariasis is still sporadically found in developed countries, with most believed to originate from zoonotic *A. suum* swine cross-transmission.

5.2. Case Study

An 8-year-old male from south Louisiana was admitted to the Children's Hospital in New Orleans with acute respiratory insufficiency with tachypnea, cough, hypoxemia, and a week-long fever. Treatment with systemic corticosteroids resulted in rapid clinical improvement. Upon further evaluation, the child was diagnosed with Loeffler syndrome based on peripheral

---

This chapter previously published as "Molecular Confirmation of Ascaris suum : Further Investigation into the Zoonotic Origin of Infection in an 8-Year-Old Boy with Loeffler Syndrome" Avery RH, Wall LA, Verhoeve VI, Gipson KS, Malone JB 2018 Vector-Borne Zoonotic Dis. 18:638–640. doi:10.1089/vbz.2018.2306, is reprinted here by permission of Mary Ann Liebert, Inc.

eosinophilia (39%), pulmonary eosinophilia by bronchoalveolar lavage (86%), diffuse reticulonodular lung opacities, mixed obstructive and restrictive pulmonary function pattern, profoundly elevated serum *Ascaris*-specific IgE detected via the ImmunoCAP assay, performed by the ARUP national reference laboratory, and a four-fold increase in total serum IgE (3,480 to 11,500 IU/mL) over a four-day period (Gipson et al. 2016). *Strongyloides* was excluded using a *Strongyloides*-specific IgG ELISA also performed by the ARUP national reference laboratory and *Toxocara* infection was excluded through a *Toxocariasis* Enzyme Immunoassay performed by the Centers for Disease Control and Prevention (CDC). The CDC also tested for *Baylisascaris procyonis*-specific antibodies using an experimental immunoblot test, and the results were negative. Bacterial and fungal cultures and stains from a bronchoalveolar lavage, fungal serum markers, viral cultures, and a large viral PCR panel excluded several other medical disorders caused by bacteria and fungi such as *Legionella*, tuberculosis, and histoplasmosis. There also were no new medications or exposures that could have caused an allergic reaction. One dose of albendazole 400 mg, as recommended by the CDC, was administered to treat the presumed *Ascaris* infection.

Patient history revealed he had never travelled outside of the United States. He lived on residential property in south Louisiana that had a small hog farm behind the main residence. His duties on the farm included feeding and watering the swine, as well as cleaning out the pens. Prior to the field visit, the two concrete pens had housed up to 30 pigs, but at the time of the visit only one 4-year-old, 204 kg intact male boar remained. A separate wooden pen had also housed a sow and piglets over two years before. When the operation previously had multiple younger

pigs, the owner had dewormed them after noticing roundworms in the pig feces. No previous parasitological diagnostic testing outside of this study had been performed on the pigs.

The lack of sanitary farm conditions likely played a role in the zoonotic *Ascaris* infection. For example, a water-hose, used in the daily care of the swine and to wash pig manure out of the pens, was allowed to lie on the concrete between the two concrete pens. Additionally, an open drainage ditch, where fecal runoff drained into a septic pit, was located at the entrance of the pens. No protective clothing and gloves were used, nor a hand sanitation station provided near the enclosures. The patient also displayed severe onychophagia (fingernail biting), and combined with the lack of proper farm sanitation, lead to the suspicion that infection was established via hand-to-oral contact while working, and the infective agent was zoonotic *Ascaris suum* acquired from either the sewage runoff or the surrounding infected soil.

To confirm the suspected etiology and zoonotic swine origin of the boy's infection, a laboratory investigation to the patient's family farm was initiated by the Louisiana Animal Disease Diagnostic Laboratory (LADDL). Fecal samples were obtained from the patient four months-post albendazole, the patient's sibling, and the boar. These were analyzed using a double-spin centrifugal flotation method (Smith et al. 2007) with $ZnSO_4$ and Sheather's sugar flotation media for the human samples and $MgSO_4$ for the swine sample. The boar sample revealed one *Ascaris* egg. No *Ascaris* eggs were found in either human sample, although *Enterobius vermicularis* eggs were found in both. The patient had been treated with albendazole prior to fecal sample examination.

To determine if infective *Ascaris* eggs of swine origin were present in the environment, soil sample transects were taken from drains surrounding the holding pens and an unfiltered

septic tank drainage ditch adjacent to the holding pens. Soil samples were processed using a modified egg extraction technique (Dunsmore et al. 1984). The extraction produced a large number of larvated, infective-stage *Ascaris* eggs. The recovered eggs were then extracted for PCR analysis.

PCR analysis of the *Ascaris* nuclear ribosomal DNA sequences spanning the first internal transcribed spacer (ITS-1) (accession no. AJ000896), (Zhu et al. 1999), was used for speciation. This segment contains polymorphic *HaeIII* restriction sites (GGCC) and has been used to differentiate *A. lumbricoides* sequences, which have been shown to contain one restriction site, from *A. suum* sequences, which generally contain two sites.

Forward (5'-TGTAATAGCAGTCGGCGGTT-3') and the reverse (5'-AACCCGATGGCGCAATGT-3') primers were created to amplify a 356 bp segment containing these restriction sites (LA_Soil-1). While not an absolute diagnostic marker (Leles et al. 2012), *HaeIII* polymorphisms are the best single biomarker to aid in differentiating the two species (Arizono et al. 2010). Analysis of the LA_Soil-1 (accession no. MG012802) PCR product revealed two *HaeIII* restriction sites, indicating that the soil eggs were *A. suum* (Figure 5.11). LA_Soil-1 also revealed two additional bps, G and C, at positions 110 and 111 respectively, compared to the Zhu sequences (Zhu et al. 1999). A second replicate, LA_Soil-2 (accession no. MG012803), displayed a T deletion at position 33, but was otherwise identical to LA_Soil-1.

5.3. Conclusions

The patient's *Ascaris*-specific IgE, his duties working with the swine, the lack of protective equipment and sanitary farm operation, the patient's onychophagia, demonstration of infective

eggs in the soil, and the subsequent DNA analysis, all indicate the patient had an *A. suum* soil-transmitted infection. Human *Ascaris* and other soil-transmitted helminth infections were once

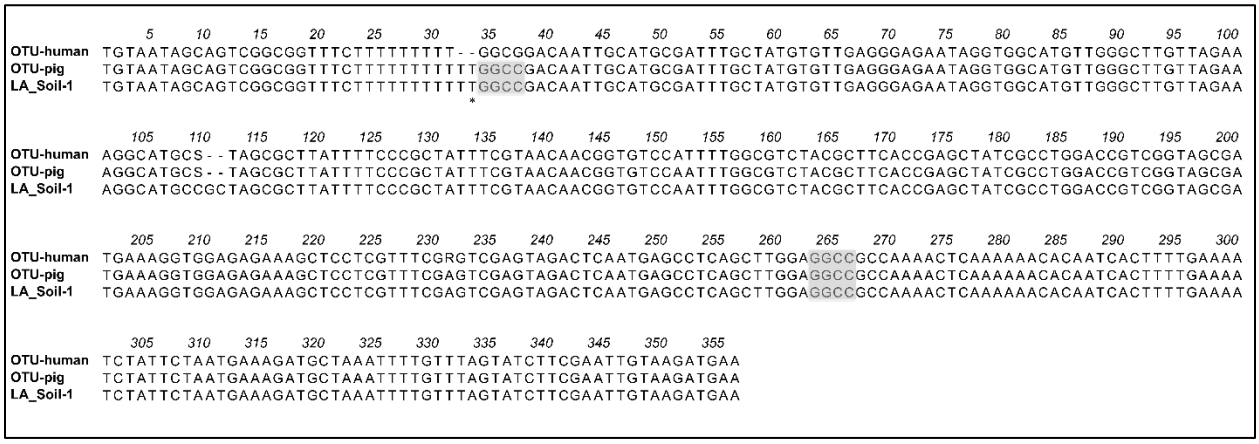

Figure 5.1. Partial *Ascaris* ITS-1 alignments comparing OTU-Pig, OTU-Human, [AJ000895, AJ000896 (Zhu et al. 1999)] and our LA_Soil-1 alignment (MG012802). The *HaeIII* binding sites are highlighted. * Indicates T deletion found in LA_Soil-2 (MG012803)

endemic throughout southern and Appalachian regions of the United States (Starr and Montgomery 2011). Improvements in education, sanitation, and hygiene practices have greatly reduced infections, but they still occasionally occur in the United States and other developed countries (Nejsum et al. 2005; Arizono et al. 2010; Nejsum et al. 2012; Miller et al. 2015; McKenna et al. 2017).

Young children in rural settings and those with direct or indirect exposure to swine and swine manure are at increased risk for zoonotic *A. suum* infections (Nejsum et al. 2005; Miller et al. 2015). We posit that many zoonotic *Ascaris* infections in the United States remain undetected in these higher-risk groups. A recent report (Miller et al. 2015) about human ascariasis on small-scale Maine farms demonstrates the risk of zoonotic *Ascaris* infections in small farm environments. This risk is compounded with improper farm management and unsanitary conditions, as was observed in the current study.

Another factor that may increase risk of ascariasis in the southern United States is the explosive increase in feral hog populations. Feral hogs in Louisiana harbor heavy parasites burdens, including *Ascaris*, and are considered a pest animal in southern states (McClure et al. 2015). Work conducted in the LADDL Parasitology Section found a 31.5% *Ascaris* prevalence rate in feral hogs hunted on Louisiana public lands from the years 2014-2015 (B. Delcambre, unpub. data). Feral swine may interact with their domestic counterparts, exposing previously treated swine as well as humans to *Ascaris* and other parasites. In this study, the farm owner noted that feral swine were previously sighted passing through the farm site.

This case provides evidence that zoonotic *A. suum*, while rare, continues to occur within the United States, particularly in small-scale hog farming operations. Lack of proper protocols to limit zoonotic transmission in small farm operations, coupled with the explosive increase in feral swine populations in the southern United States, increases the risk of *A. suum* infections in both humans and domestic swine in the future.

5.4. References

Arizono N, Yoshimura Y, Tohzaka N, Yamada M, Tegoshi T, Onishi K, Uchikawa R. 2010. Ascariasis in Japan: is pig-derived Ascaris infecting humans? Jpn. J. Infect. Dis. 63:447–8.

Bethony J, Brooker S, Albonico M, Geiger SM, Loukas A, Diemert D, Hotez PJ. 2006. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. Lancet 367:1521–1532. doi:10.1016/S0140-6736(06)68653-4.

Dunsmore JD, Thompson RCA, Bates IA. 1984. Prevalence and survival of Toxocara canis eggs in the urban environment of Perth, Australia. Vet. Parasitol. 16:303–311. doi:10.1016/0304-4017(84)90048-7.

Gipson K, Avery R, Shah H, Pepiak D, Bégué RE, Malone J, Wall LA. 2016. Löffler syndrome on a Louisiana pig farm. Respir. Med. Case Reports 19:128–131. doi:10.1016/j.rmcr.2016.09.003.

Leles D, Gardner SL, Reinhard K, Iñiguez A, Araujo A. 2012. Are Ascaris lumbricoides and Ascaris suum a single species? Parasit. Vectors 5:42. doi:10.1186/1756-3305-5-42.

McClure ML, Burdett CL, Farnsworth ML, Lutman MW, Theobald DM, Riggs PD, Grear DA, Miller RS. 2015. Modeling and mapping the probability of occurrence of invasive wild pigs across the contiguous United States. PLoS One 10:e0133771. doi:10.1371/journal.pone.0133771.

McKenna ML, Mcatee S, Hotez PJ, Bryan PE, Jeun R, Bottazzi ME, Flowers CC, Ward T, Kraus J, Mejia R, et al. 2017. Human Intestinal Parasite Burden and Poor Sanitation in Rural Alabama. Am. J. Trop. Med. Hyg 97:1623–1628. doi:10.4269/ajtmh.17-0396.

Miller LA, Colby K, Manning SE, Hoenig D, McEvoy E, Montgomery S, Mathison B, de Almeida M, Bishop H, Dasilva A, et al. 2015. Ascariasis in humans and pigs on small-scale farms, Maine, USA, 2010-2013. Emerg. Infect. Dis. 21:332–4. doi:10.3201/eid2102.140048.

Nejsum P, Betson M, Bendall RP, Thamsborg SM, Stothard JR. 2012. Assessing the zoonotic potential of Ascaris suum and Trichuris suis: looking to the future from an analysis of the past. J. Helminthol. 86:148–155. doi:10.1017/S0022149X12000193.

Nejsum P, Parker ED, Frydenberg J, Roepstorff A, Boes J, Haque R, Astrup I, Prag J, Skov Sørensen UB, Sørensen UBS. 2005. Ascariasis is a zoonosis in denmark. J. Clin. Microbiol. 43:1142–8. doi:10.1128/JCM.43.3.1142-1148.2005.

Smith PH, Wiles SE, Malone JB. 2007. Parasitology Veterinary Diagnostic Laboratory Manual. Baton Rouge, LA: Louisiana State University.

Starr MC, Montgomery SP. 2011. Soil-transmitted helminthiasis in the United States: A systematic review - 1940-2010. Am. J. Trop. Med. Hyg. 85:680–684. doi:10.4269/ajtmh.2011.11-0214.

Zhu X, Chilton NB, Jacobs DE, Boes J, Gasser RB. 1999. Characterisation of Ascaris from human and pig hosts by nuclear ribosomal DNA sequences. Int. J. Parasitol. 29:469–478.

Chapter 6. Conclusions, Policy Recommendations, and Future Directions

In Brazil, decades of morbidity control and an increase in the overall standard of living have contributed to decreasing (<20%) STH infection prevalence throughout most of the country (Chammartin et al. 2014). The relatively low overall STH prevalence provides an opportunity to achieve STH elimination. However, to achieve this goal, the traditional MDA control programs and the reliance on passive surveillance must be replaced with more active surveillance and a response system geared towards elimination (Bergquist et al. 2015; Bergquist et al. 2017). For an effective To achieve STH elimination in Feira de Santana, Brazil we provide evidence that several changes are needed:

- adoption of sensitive and consistent diagnostic techniques to enable more accurate case-finding
- expansion of STH infection surveillance from SAC to encompass entire individual households
- implementation of cost-effective, individual household-scale resolution (<5m^2) surveillance and response mapping to guide treatment and control protocols
- modification of treatment and control protocols tailored to specific communities based on the predominant STH infection and environmental predictors of STH infection obtained through household-scale ENM modeling,

In the city of Feira de Santana, we propose that the opportunity to institute this shift towards STH elimination and implement the above suggested changes a is feasible. Our research conducted in Feira de Santana demonstrated <20% STH prevalence across the three representative communities of Maria Quiteria, CDGN, and Queimadinha, and a difference in the

predominant type of STH infection varying among the communities. We demonstrated that the qPCR diagnostic test provides comparable detection rates to Kato-Katz and identified several STH infections that were missed using Kato-Katz. While we were unable to demonstrate significantly different detection rates using the qPCR as compared to Kato-Katz method in this study, other studies by Mejia et al. (2013) and Cimino et al. (2015) have demonstrated its superior detection rates when compared to the Telemann concentration, direct wet mount smear, and the McMaster method commonly used diagnostic STH tests. The qPCR test provides the additional benefit of detecting multiple gastrointestinal parasites using a single test procedure, saving time and manpower that would otherwise require the use of multiple different diagnostic tests.

Our recommendation to the Feira de Santana MOH is to begin implementation of the qPCR diagnostic to replace both the Kato-Katz thick smear and their current protozoal diagnostic test(s). While the upfront cost of a quantitative PCR system is expensive, the long-term and downstream cost savings become apparent when evaluated within the context of the money and time saved due to increased STH treatment and eventual elimination, as well as its monetary impact on other gastrointestinal parasites. Performing the qPCR reaction on fecal samples is cost-effective, with the overall cost of analyzing fecal samples for gastrointestinal parasites at <$1.00 per sample (Mejia et al. 2013). To test for all the gastrointestinal parasites analyzed using qPCR would cost $2.60 per sample (Basuni et al. 2012). The ability to test for multiple parasites with a single test eliminates the additional time and manpower required using microscopy diagnostic tests. Testing would benefit from requiring only a very single, small amount of fecal sample and only a single testing instance.

Our study in Feira de Santana revealed that adults within households serve as reservoirs for STH infection and likely contribute to reinfection of the children in the household. To eliminate STH, adult infections must also be detected and treated and therefore we recommend that the MOH move away from school-based sampling and instead institute whole household sampling. We also recommend the use of very-high resolution <5m^2 satellite data to allow predictive STH niche modeling focused on the individual households and their surrounding habitat. Our research demonstrated that the VHR satellite GE1 provides the resolution required to evaluate the ecological niche of each STH at this high-resolution household scale within our three communities that would have otherwise been impossible to accurately model using the traditional Landsat 8 satellite (30m^2). GE1 also provided comparable household ENMs when compared to the VHR satellite WV2 and provides the added benefits of being cheaper and having a larger image archive.

Differences between each community in both the predominant STH type and the corresponding environmental predictors demonstrates the need to evaluate each community independently and craft individualized elimination interventions. We recommend that the Feira de Santana, MOH continue to build on the foundation of our STH GIS using the GE1 satellite to implement their surveillance and response system. This active system would target specific communities within the municipality, as opposed to approaching the municipality as a single unit. The communities in this study should be sampled in the areas predicted to be highly suitable for STH infection and those households targeted for treatment. Follow-up sampling should take place to test whether the treatment of these high-risk areas, and particularly the treatment of heavily parasitized individuals, leads to an overall decrease or even possibly interruption of

transmission of STH infection. The surveillance and response GIS should be continually updated with the new sampling results, allowing for continual updating and optimization of the system so it more accurately predicts STH niches. If the new interventions proved successful, the next step would be to choose new communities to enact this elimination approach based on the likelihood of higher STH prevalence, using the three original test communities ENMs as the basis to help selection. We recommend that the surveillance and response system incorporate other diseases of public health concern, such as schistosomiasis and leishmaniasis, enabling them to be more efficient and cost-effective. This provides an opportunity to evaluate and treat multiple diseases that impact communities simultaneously.

Our research provides the groundwork from which an STH elimination surveillance and response system can be built for the city of Feira de Santana. With the implementation of the qPCR diagnostic, the change of sampling focus towards entire households, the ability to model STH ecological niches accurately on a household-habitat scale of $<5m^2$, and the application of specific elimination protocols altered to fit each specific community, we propose that Feira de Santana can ultimately interrupt STH transmission and eliminate the infections within the municipality, eliminating STH as a public health problem.

6.1. References

Basuni M, Mohamed Z, Ahmad M, Zakaria NZ, Noordin R. 2012. Detection of selected intestinal helminths and protozoa at Hospital Universiti Sains Malaysia using multiplex real-time PCR. Trop. Biomed. 29:434–42.

Bergquist R, Yang GJ, Knopp S, Utzinger J, Tanner M. 2015. Surveillance and response: Tools and approaches for the elimination stage of neglected tropical diseases. Acta Trop. 141:229–234. doi:10.1016/j.actatropica.2014.09.017.

Bergquist R, Zhou XN, Rollinson D, Reinhard-Rupp J, Klohe K. 2017. Elimination of schistosomiasis: The tools required. Infect. Dis. Poverty 6:158. doi:10.1186/s40249-017-0370-7.

Chammartin F, Guimarães LH, Scholte RGC, Bavia ME, Utzinger J, Vounatsou P. 2014. Spatio-temporal distribution of soil-transmitted helminth infections in Brazil. Parasites and Vectors 7:440. doi:10.1186/1756-3305-7-440.

Cimino RO, Jeun R, Juarez M, Cajal PS, Vargas P, Echazú A, Bryan PE, Nasser J, Krolewiecki A, Mejia R. 2015. Identification of human intestinal parasites affecting an asymptomatic peri-urban Argentinian population using multi-parallel quantitative real-time polymerase chain reaction. Parasit. Vectors 8:380. doi:10.1186/s13071-015-0994-z.

Mejia R, Vicuña Y, Broncano N, Sandoval C, Vaca M, Chico M, Cooper PJ, Nutman TB. 2013. A novel, multi-parallel, real-time polymerase chain reaction approach for eight gastrointestinal parasites provides improved diagnostic capabilities to resource-limited at-risk populations. Am. J. Trop. Med. Hyg. 88:1041–1047. doi:10.4269/ajtmh.12-0726.

Appendix A. Louisiana State University IRB Approval

ACTION ON PROTOCOL APPROVAL REQUEST

Institutional Review Board
Dr. Dennis Landin, Chair
130 David Boyd Hall
Baton Rouge, LA 70803
P: 225.578.8692
F: 225.578.5983
irb@lsu.edu | lsu.edu/irb

TO: John Malone
Pathobiological Sciences

FROM: Dennis Landin
Chair, Institutional Review Board

DATE: January 28, 2016

RE: IRB# 3633

TITLE: Use of Ecological Niche Modeling and Molecular Diagnostics for Community-Based Elimination of Soil-Transmitted Helminthiasis in Bahia, Brazil

New Protocol/Modification/Continuation: New Protocol

Review type: Full ___ Expedited X **Review date:** 1/28/2016

Risk Factor: Minimal ___X___ Uncertain _____ Greater Than Minimal_____

Approved_____X_____ **Disapproved**_____

Approval Date: 1/28/2016 **Approval Expiration Date:** 1/27/2017

Re-review frequency: (annual unless otherwise stated)

Number of subjects approved: Depends

LSU Proposal Number (if applicable):

Protocol Matches Scope of Work in Grant proposal: (if applicable) _____

By: Dennis Landin, Chairman _____

PRINCIPAL INVESTIGATOR: PLEASE READ THE FOLLOWING –
Continuing approval is CONDITIONAL on:

1. Adherence to the approved protocol, familiarity with, and adherence to the ethical standards of the Belmont Report, and LSU's Assurance of Compliance with DHHS regulations for the protection of human subjects*
2. Prior approval of a change in protocol, including revision of the consent documents or an increase in the number of subjects over that approved.
3. Obtaining renewed approval (or submittal of a termination report), prior to the approval expiration date, upon request by the IRB office (irrespective of when the project actually begins); notification of project termination.
4. Retention of documentation of informed consent and study records for at least 3 years after the study ends.
5. Continuing attention to the physical and psychological well-being and informed consent of the individual participants, including notification of new information that might affect consent.
6. A prompt report to the IRB of any adverse event affecting a participant potentially arising from the study.
7. Notification of the IRB of a serious compliance failure.
8. **SPECIAL NOTE: When emailing more than one recipient, make sure you use bcc.**

**All investigators and support staff have access to copies of the Belmont Report, LSU's Assurance with DHHS, DHHS (45 CFR 46) and FDA regulations governing use of human subjects, and other relevant documents in print in this office or on our World Wide Web site at http://www.lsu.edu/irb*

Appendix B. First Page of Approval From Brazilian National Commission of Research Ethics

COMISSÃO NACIONAL DE ÉTICA EM PESQUISA

PlataForma Brasil

PARECER CONSUBSTANCIADO DA CONEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: MONITORAMENTO E SISTEMA DE RESPOSTA PARA GEO-HELMINTOS USANDO MODELAGEM DE NICHO ECOLÓGICO E DIAGNÓSTICO MOLECULAR

Pesquisador: Ryan Avery

Área Temática: Pesquisas com coordenação e/ou patrocínio originados fora do Brasil, excetuadas aquelas com copatrocínio do Governo Brasileiro;

Versão: 2

CAAE: 54801816.0.1001.0053

Instituição Proponente: Louisiana State University

Patrocinador Principal: Louisiana State University

DADOS DO PARECER

Número do Parecer: 1.718.092

Apresentação do Projeto:

INTRODUÇÃO

As Geo-helmintoses são causadas principalmente pelo Trichuris trichiura, Ascaris lumbricoides, Necator americanus e Ancylostoma duodenale. Acredita-se que atualmente os Geo-helmintos infectam mais de 1 bilhão de pessoas e existem mais outros bilhões em risco de adoecer, especialmente aqueles que vivem em climas quentes e úmidos. A Organização Mundial da Saúde (OMS) considera as Geo-helmintoses como doenças tropicais negligenciadas (DTN) que afetam principalmente as comunidades mais vulneráveis, aquelas que são empobrecidas e vivem em precárias condições sanitárias. As Geo-helmintoses podem causar problemas graves de morbidade nos seres humanos, e, portanto, são avaliadas em termos do número de anos de vida perdidos por incapacidade (DALY). Questões de morbidade incluem perdas cognitivas e no crescimento físico, aptidão física prejudicada, e impactos negativos no desempenho escolar. Normalmente, infecções por geo-helmintos são mais intensas e têm o maior impacto em crianças em idade pré-escolar e idade escolar e, portanto, programa de administração de tratamento em massa deve focar este grupo de indivíduos. No entanto, as infecções por parasitas quando continuam altas na idade adulta podem causar complicações graves em mulheres grávidas, por exemplo. Embora frequentemente as três doenças sejam agrupadas como geo-helmintoses, elas variam muito entre

Endereço: SEPN 510 NORTE, BLOCO A 3º ANDAR, Edifício Ex-INAN - Unidade II - Ministério da Saúde
Bairro: Asa Norte **CEP:** 70.750-521
UF: DF **Município:** BRASILIA
Telefone: (61)3315-5878 **E-mail:** conep@saude.gov.br

Appendix C. Family Questionnaire in English

Family Questionnaire

1. Locality_____
2. Household number (Family ID)_____
3. GPS coordinates_____
4. How many people live in the household? _____
5. How many females live in the household? _____
6. How many males live in the household? _____
7. How many people in the household ages: 0-10 _____
   11-20 _____
   21-30 _____
   31-40 _____
   41-50 _____
   51-60 _____
   61-70 _____
   71-80 _____
   81-89 _____

Urban Zone ( )
Peri-Urban Zone ( )
Rural Zone ( )

8. How many people in the household attend elementary school? _____
9. How many people in the household attend high school? _____
10. How many people in the household are regularly employed? _____
11. How many rooms in the house? _____
12. What is your main source of drinking water?
(1) Piped water (2) Lake/river/stream (3) Well (4) Rainwater (5) Other_____
13. What is your main source of daily general use water?
(1) Piped water (2) Lake/river/stream (3) Well (4) Rainwater (5) Other_____
14. Is your drinking water filtered? Y N
15. Do you have a bathroom in the house? Y N
16. If yes, do you have a flushing toilet? Y N
17. If no, do you have a bathroom outside of the house? Y N
18. Is there a hand washing faucet station in the home? Y N
19. Does your home have electricity? Y N
20. What flooring material is in the home? Wood Cement Ceramic Earth
Other_____
21. What roofing material is on the home? Metal Wood/Shingle Thatch/Palm Roof Tile
Slab Slab/Plaster Other_____
22. What is the main way you dispose of your garbage?
(1) Burning (2) Left in open air (3) Collected by municipality (4) Other_____
23. Do you raise and/or own swine? Y N
24. If yes, how far away are the swine to your house? _____
25. Do any of your neighbors raise and/or own swine? Y N
26. If yes, how far away are the swine to your house? _____

Appendix D. Family Questionnaire in Portuguese

Apêndice 1 - Questionário de Campo (Família)

Zona Urbana ( )
Zona Peri-urbana ( )
Zona Rural ( )

1. Localidade _____
2. Número da Casa (ID) _____
3. Coordenadas de GPS _____
4. Quantas pessoas vivem na residência? _____
5. Quantas mulheres vivem na residência? _____
6. Quantos homens vivem na residência? _____
7. Quantas pessoas vivem na residência com faixa etária:

0-10 _____ 51-60 _____
11-20 _____ 61-70 _____
21-30 _____ 71-80 _____
31-40 _____ 81-89 _____
41-50 _____ Acima de 89_____

8. Quantas pessoas na residência possuem ensino fundamental? _____
9. Quantas pessoas na residência possuem ensino médio? _____
10. Quantas pessoas na residência trabalham? _____
11. Quantos cômodos a residência possui? _____
12. Qual é a fonte principal de água para consumo (beber)?

(1) Água encanada (2) Lago/rio (3) Poço (4) Água de chuva (5) Outra fonte_____

13. Qual é a fonte principal de água de uso diário?

(1) Água encanada (2) Lago/rio (3) Poço (4) Água de chuva (5) Outra fonte_____

14. A água de beber é filtrada? ( ) Sim ( ) Não
15. Você possui banheiro dentro da residência? ( ) Sim ( ) Não
16. Se sim, a privada possui descarga? ( ) Sim ( ) Não
17. Se não, você possui um banheiro fora da residência? ( ) Sim ( ) Não
18. Existe algum local com torneira para lavagem das mãos na residência? ( ) Sim ( ) Não
19. A sua residência possui energia elétrica? ( ) Sim ( ) Não
20. Qual o tipo de piso/chão da residência?

(1) Madeira (2) Cimento (3) Chão batido (4) Cerâmica (5) Outro_____

21. Qual o tipo de telhado da residência?

(1) Metal (2) Madeira (3) Sapé/Palma (4) Telha (5) Laje (6) Laje/Gesso (7) Outro_____

22. Qual é o principal destino do lixo?

(1) Queimado (2) Abandonado (3) Coleta domiciliar (4) Outro_____

23. Você cria ou possui suínos? ( ) Sim ( ) Não
24. Se sim, qual a distância do suíno/criadouro para a residência? _____
25. Algum dos seus vizinhos cria ou possuem suínos? ( ) Sim ( ) Não
26. Se sim, qual a distância desses suínos para a sua residência? _____

Appendix E. Individual Questionnaire in English

Individual Questionnaire

1. Locality_____ **Urban Zone ( )**
2. Personal number (ID) _____ **Peri-Urban Zone ( )**
3. Sample Number _____ **Rural Zone ( )**
4. GPS Coordinates _____
5. Age _____
6. Sex M F
7. Have you heard of *Ascaris*, hookworm, or *Schistosoma* worms? Y N
8. Do you know how each one is transmitted?
   - *Ascaris* Y N
   - Hookworm Y N
   - *Schistosoma* Y N
9. Have you been diagnosed with one or more of these worms? Y N
   Past or currently infected? _____
10. If yes, which one(s)? (Circle all that apply) (1) *Ascaris* (2) hookworm (3) *Schistosoma*
11. Have you been treated for any of these worms?
    If so, time since last treatment? _____
12. Do you normally wear shoes outside? Always Sometimes Rarely Never
13. If you wear shoes outside, what is the type you most often wear?
    Closed toed Open toed
14. Do you have relatives in a rural community that you visit? (For peri-urban and urban residents)
    Y N
15. If yes, where is it and how often do you visit them? _____
16. Do you work with swine? Y N
17. If yes, do you clean your shoes after working with swine? Y N
18. Does anyone else in your family work with swine? Y N
19. If yes, do they clean their shoes off after working with swine? Y N
20. What is the highest level of your education?
    (1) 1st to 4th not completed
    (2) 4th completed
    (3) 5th to 8th grade not completed
    (4) Elementary school completed
    (5) High school incomplete
    (6) High school completed
    (7) Higher education completed
21. What is the highest level of your head of household's education?
    (1) 1st to 4th not completed
    (2) 4th completed
    (3) 5th to 8th grade not completed
    (4) Elementary school completed
    (5) High school incomplete
    (6) High school completed
    (7) Higher education completed

Appendix F. Individual Questionnaire in Portuguese

Apêndice 2 - Questionário de Campo (Individual)

Zona Urbana ( )
Zona Peri-urbana ( )
Zona Rural ( )

1. Localidade _____
2. Número da Casa _____
3. Número da Amostra _____
4. Coordenadas de GPS _____
5. Idade _____
6. Sexo (1) Masculino (2) Feminino
7. Você tem algum conhecimento sobre os vermes :

Lombriga (*Ascaris*), Amarelão (*Ancylostoma*), Barriga d'água (*Schistosoma*)? ( ) Sim ( )Não

8. Você tem conhecimento sobre como cada um desses vermes é transmitido?

Ascaris (lombriga) ( ) Sim ( )Não
Ancylostoma (amarelão) ( ) Sim ( )Não
Schistosoma (barriga d'água) ( ) Sim ( ) Não

9. Você já foi diagnosticado com um ou mais destes vermes? ( ) Sim ( )Não
10. Infectado no passado ou infectado atualmente? _____
11. Se sim, qual (is)? (Circule os que se aplicam) (1) Lombriga (2) Amarelão (3) Barriga d´água
12. Você já foi tratado para algum desses vermes? ( ) Sim ( )Não
13. Se sim, quando foi o último tratamento? _____
14. Você geralmente usa sapatos quando está fora da residência?

(1) Sempre (2) Algumas vezes (3) Raramente (4) Nunca

15. Se você usa sapatos quando está fora da residência, qual o tipo de sapato que você usa com mais frequência?

(1) Sapato fechado (2) Sandália

16. Você tem parentes em comunidade rural? (Para os moradores peri-urbano e urbano)

( ) Sim ( )Não

17. Se sim, qual localidade e com que frequência os visita?_____
18. Você trabalha com suínos? ( ) Sim ( )Não
19. Você limpa seus sapatos depois de trabalhar com suínos? ( ) Sim ( )Não
20. Alguém em sua família trabalha com suínos? ( ) Sim ( )Não
21. Eles limpam os sapatos depois de trabalhar com os suínos? ( ) Sim ( )Não
22. Qual o seu nível de escolaridade?
    (1) 1º ao 5º ano incompleto (Fundamental I)
    (2) 5º ano completo (Fundamental I)
    (3) 6º ao 9º ano incompleto (Fundamental II)
    (4) Ensino Fundamental II completo
    (5) Ensino Médio incompleto
    (6) Ensino Médio completo
    (7) Ensino Superior incompleto
    (8) Ensino superior completo
    (9) Não alfabetizado
23. Qual o nível de escolaridade mais alto do companheiro(a) do chefe da família?
    (1) 1º ao 5º ano incompleto (Fundamental I)
    (2) 5º ano completo (Fundamental I)
    (3) 6º ao 9º ano incompleto (Fundamental II)
    (4) Ensino Fundamental II completo
    (5) Ensino Médio incompleto
    (6) Ensino Médio completo
    (7) Ensino Superior incompleto
    (8) Ensino superior completo
    (9) Não alfabetizado

Appendix G. Informed Consent in English

APPENDIX 3
INFORMED CONSENT

To Whom It May Concern,

We are conducting a survey "A Monitoring and Response System for Geo-Helminths Using Ecological Niche Modelling and Molecular Diagnostics" in order to determine the prevalence of geohelminths, parasitic infections in people, caused by Ascaris lumbricoides (Roundworm), Ancylostoma/Necator spp. (Hookworm), and Trichuris trichiura (Whipworm) in urban and rural areas in the municipality of Feira de Santana. The research coordinator is Ryan Avery and works in collaboration with Dr. Aristeu Vieira da Silva and Dr. Simone Souza de Oliveira. You and your family are being invited to participate in this research, where stool samples will be collected from family members in all age groups. This study intends to follow the ethical principles for research involving human beings, avoiding damage/harm to participants, and will be conducted on the Campus of the State University of Feira de Santana, Clinical and Parasitological Analysis Lab (LAC) located in room 30, building LABIO. This is where we will conduct stool tests in collaboration of other lab personnel. Stool collection will be performed by the participant (yourself) in sterile fecal collection containers that you will receive in advance, and the research team will give you instructions on how to perform the collection. If the material is insufficient or if there are any problems in the original collection, we are responsible for informing you and a new collection and test can be performed. It is important to note that the techniques of collecting, storing, transporting and handling are performed using strict biosecurity criteria. This study also intends to establish a relationship between the results obtained and the socio-economic, environmental and health conditions of the areas studied, and this information will be obtained during an interview with the head of the family and/or other study participants. Research involving human beings can cause risks to the subjects surveyed, however this study may only cause some discomfort during the collection of the biological material. To this end, the team was instructed in advance to minimize these or any other risks that may arise, and in addition the research team will be available for the any possible eventualities and will seek to rectify any issues that may occur on the collection of these samples and their use in this research. Participation is voluntary and we will be at your disposal for any clarification on the research and you will have every right to drop out if you do not feel properly satisfied. All your answers to the questions asked will be kept secret, in addition to the results of laboratory tests, and will never be submitted anywhere without results being anonymous. The interview, which will be conducted on the day of delivery of fecal collection material for laboratory examination, will be used only in this study. After the house visit is conducted, all forms will be stored under the responsibility of the Coordinator of the study for a period of five years and stored in LAC-UEFS (LABIO 30 Room) and may not be used in any other research. The biological materials, after being analyzed by the tests specified in the Protocol, will be disposed of following appropriate biosecurity standards. The results of this research may help identify cases of diseases caused by parasites, and you and your family will be provided with the opportunity receive appropriate treatment from health service. Any children under the age of 18 years shall participate only with the authorization of a parent or guardian, and after signing the Term of Free and Informed Consent. The results will also be used for the doctoral thesis of the project Coordinator, and may be presented in summary form at scientific events, articles in scientific journals and research reports After the analysis of stool samples, the results will be delivered to the people responsible for the subjects in the study and you and your family will be directed to the Basic Health Unit in their area with the necessary recommendations, if indicated, so that you and your family can receive appropriate assistance from the health service. If you agree to participate, you must sign this term in two copies of equal content. A copy of it will be with you and the other with the responsible researchers. For your cooperation, you will not receive any cash value, but will have the guarantee that all expenses necessary for the implementation of this research are not your responsibility. If there is a case of injury you may be compensated where appropriate. You can contact the project team at any time by phone: Ryan Avery; (71) 99100-4840, email: ravery3@lsu.edu; Profa. Simone Souza de Oliveira, e-mail: simone23_oliveira@yahoo.com.br and Prof. Aristeu Vieira da Silva, email: aristeuvsilva@uefs.br or by phone (75) 3161-8796 (LAC-UEFS), (75) 3161-8019 (Department of biological sciences). If you have questions related to ethical questions you may call the CEP-UEFS (75) 3161-8067 or contact them via email at cep@uefs.br. The address of the researchers is UEFS-Highway Transnordestina, s/n, Bairro Novo Horizonte – 44,036-900 – Feira de Santana-BA-Department of Biological Sciences – Laboratory of Clinical Analyses – Building LABIO, Room 30.

Feira de Santana – Bahia, ___/___/___ _____

Ryan Avery – Research Project Coordinator
(75) 99100-4840 - LAC (UEFS) (75) 3161-8796

Name: _____

Signature: _____

Appendix H. Informed Consent in Portuguese

APÊNDICE 3

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Senhor(a),

Estamos realizando uma pesquisa **"Monitoramento e Sistema de Resposta para Geo-Helmintos Usando Modelagem de Nicho Ecológico e Diagnóstico Molecular"** com o objetivo de determinar a prevalência de geohelmintos, infecções parasitárias em pessoas, causadas por Ascaris (Lombriga), Ancylostoma (Amarelão) e Trichuris (Tricuríase) em áreas periféricas no município de Feira de Santana, tendo como responsável Dr. Ryan Arvery e colaboração dos professores Drª Aristeu Vieira da Silva e Prof. Drª Simone Souza de Oliveira. Você e sua família estão sendo convidados a participar desta pesquisa, onde serão coletadas amostras de fezes de cada componente da família em todas as faixas etárias. Este estudo pretende seguir os princípios éticos de pesquisa envolvendo seres humanos, evitando danos/agravos aos participantes e será realizado no Campus da Universidade Estadual de Feira de Santana, Laboratório de Análise Clinicas e parasitológicas (LAC) localizado no LABIO sala 30 que já vem realizando exames de fezes, possuindo estrutura física e de pessoal adequadas para realização dos mesmos. A coleta de fezes será realizada por você mesmo e para isso você receberá previamente um coletor de fezes esterilizado e, a equipe de pesquisadores lhe dará as instruções para a realizar a coleta. Caso o material seja insuficiente ou ocorra qualquer problema na execução do exame, nova coleta poderá ser realizada, devendo o responsável informar e marcar com antecedência o novo procedimento. É importante ressaltar que as técnicas de coleta, armazenamento, transporte e manipulação são executadas com rigoroso critério de biossegurança. Este estudo também pretende estabelecer uma relação entre os resultados obtidos e as condições socioeconômicas, ambientais e sanitárias das áreas estudadas, obtidas durante a entrevista com o chefe da família e/ou responsável, registrada em formulário próprio. As pesquisas envolvendo seres humanos são passíveis de riscos para os sujeitos pesquisados, porém este estudo pode despertar em você algum tipo de desconforto durante a coleta de dados e do material biológico. Para tanto, a equipe foi treinada previamente visando minimizar estes ou outros riscos inerentes que possam surgir, bem como a coordenação do estudo e a equipe executora estará à disposição para as possíveis eventualidades e buscará reparar/indenizar prejuízos que ocorrerem por conta da coleta destas amostras e sua utilização nesta pesquisa. A participação é voluntária e a qualquer momento estaremos a sua disposição para qualquer esclarecimento sobre a pesquisa e você terá todo o direito de desistir dela quando não se sentir devidamente satisfeito (a) sem nenhum prejuízo para o senhor (a) e a sua família. Tudo que lhe será perguntado e todas as suas respostas serão mantidas em segredo assim como os resultados dos exames laboratoriais, e nunca serão apresentadas relacionadas com seu nome. A entrevista, que será realizada no dia da entrega do material para exame laboratorial em local reservado, será usada apenas neste estudo. Após a pesquisa, os formulários serão guardados sob a responsabilidade do coordenador do estudo por um período de cinco anos e armazenados no LAC-UEFS (LABIO Sala 30), não podendo ser utilizados em nenhuma outra pesquisa. Os materiais biológicos, após os exames especificados no protocolo, serão destinados ao descarte de amostras biológicas segundo os padrões de biossegurança. Os resultados desta pesquisa poderão ajudar a identificar os casos de doenças causadas por parasitos, e com isso propiciar a oportunidade de que você e sua família recebam os cuidados adequados por parte do serviço de saúde, e com isso, melhorar a saúde de sua família. Quaisquer crianças e jovens menores de 18 anos só participarão com a autorização dos pais ou dos responsáveis, e após assinarem o Termo de Assentimento Livre e Esclarecido. Os resultados também serão utilizados com finalidade científica na produção da Tese de Doutorado do coordenador do projeto, podendo ser apresentados na forma de resumo em eventos científicos, artigos em revistas cientificas e relatórios de pesquisa. Após as análises das amostras de fezes, os resultados serão entregues nos próprios domicílios e as pessoas serão orientadas a procurar a Unidade Básica de Saúde da sua área de abrangência para as recomendações necessárias, caso seja indicado, para que as famílias recebam a assistência adequada por parte do serviço de saúde. Se você concordar em participar, deverá assinar este termo em duas vias de igual teor. Uma cópia dele ficará com você e a outra com os pesquisadores responsáveis. Por sua colaboração, você não receberá qualquer valor em dinheiro, mas terá a garantia de que todas as despesas necessárias para a realização desta pesquisa não serão de sua responsabilidade havendo você será compensado. Verificado qualquer dano você poderá ser indenizado se for o caso. Você poderá entrar em contato com a equipe do projeto a qualquer momento que julgar necessário pelos telefones: Ryan Avery, Profa. Simone Souza de Oliveira e Prof. Aristeu Vieira da Silva pelos telefones: Ryan Avery; (71) 99100-4840, e-mail: ravery3@lsu.edu; Profa. Simone Souza de Oliveira, e-mail: simone23_oliveira@yahoo.com.br e Prof. Aristeu Vieira da Silva, e-mail: aristeuvsilva@uefs.br ou pelos telefones (75) 3161-8796 (LAC-UEFS), (75) 3161-8019 (Departamento de Ciências Biológicas). Em caso de dúvidas relacionadas a questões éticas você pode ligar para o CEP-UEFS (75) 3161-8067 ou manter contato pelo e-mail cep@uefs.br. Endereço da UEFS – Rodovia Transnordestina, s/n, Bairro Novo Horizonte – 44.036-900 – Feira de Santana – BA - Departamento de Ciências Biológicas – Laboratório de Análises Clinicas - LABIO, Sala 30.

Feira de Santana – Bahia, ___/___/___ _____

Ryan Avery - Coordenador do Projeto de Pesquisa

(75) 99100-4840 - LAC (UEFS)(75) 3161-8796

Nome: _____

Assinatura: _____

Appendix I. Parental Consent Form in English

APPENDIX 4

Informed Consent (Parent or Legal Guardian)

To Whom It May Concern,

We are conducting a survey "A Monitoring and Response System for Geo-Helminths Using Ecological Niche Modelling and Molecular Diagnostics" in order to determine the prevalence of geohelminths, parasitic infections in people, caused by *Ascaris lumbricoides* (Roundworm), *Ancylostoma/Necator spp.* (Hookworm), and *Trichuris trichiura* (Whipworm) in urban and rural areas in the municipality of Feira de Santana. The research coordinator is Ryan Avery and works in collaboration with Dr. Aristeu Vieira da Silva and Dr. Simone Souza de Oliveira. I'm inviting you to authorize the participation of your minor(s) in this research, where stool samples will be collected. This study intends to follow the ethical principles for research involving human beings, avoiding damage/harm to participants, and will be conducted on the Campus of the State University of Feira de Santana, Clinical and Parasitological Analysis Lab (LAC) located in room 30, building LABIO. This is where we will conduct stool tests in collaboration of other lab personnel. Stool will be collected from both minors and those responsible for them and to achieve this you will receive in advance a sterile container for feces and the research team will give you the instructions on how to perform the collection. If the material is insufficient or if there are any problems in the original collection, we are responsible for informing you and a new collection and test can be performed. It is important to note that the techniques of collecting, storing, transporting and handling are performed using strict biosecurity criteria. This study also intends to establish a relationship between the results obtained and the socio-economic, environmental and health conditions of the areas studied, and this information will be obtained during an interview with the head of the family and/or other study participants. Research involving human beings can cause risks to the subjects surveyed, however this study may only cause some discomfort during the collection of the biological material. To this end, the team was instructed in advance to minimize these or any other risks that may arise, and in addition the research team will be available for the any possible eventualities and will seek to rectify any issues that may occur on the collection of these samples and their use in this research. Minor(s) participation is voluntary, if they are authorized by a parent/guardian, and we will be at your disposal for any clarification on the research and you will have every right to drop out if you do not feel properly satisfied. All your answers to the questions asked will be kept secret, in addition to the results of laboratory tests, and will never be submitted anywhere without results being anonymous. The interview, which will be conducted on the day of delivery of fecal collection material for laboratory examination, will be used only in this study. After the house visit is conducted, all forms will be stored under the responsibility of the Coordinator of the study for a period of five years and stored in LAC-UEFS (LABIO 30 Room) and may not be used in any other research. The biological materials, after being analyzed by the tests specified in the Protocol, will be disposed of following appropriate biosecurity standards. The results of this research may help identify cases of diseases caused by parasites, and you and/or your minor(s) will be provided with the opportunity receive appropriate treatment from health service. Any children under the age of 18 years shall participate only with the authorization of a parent or guardian, and after signing the Term of Free and Informed Consent. The results will also be used for the doctoral thesis of the project Coordinator, and may be presented in summary form at scientific events, articles in scientific journals and research reports. After the analysis of stool samples, the results will be delivered to the people responsible for the subjects in the study and will be directed to the basic Health Unit in their area with the necessary recommendations, if indicated, so that the minor(s) receive appropriate assistance by the health service. If you authorize the participation of the minor, you must sign this term in two copies of equal content. A copy of it will be with you and the other with the responsible researchers. For your cooperation, you will not receive any cash value, but will have the guarantee that all expenses necessary for the implementation of this research are not your responsibility. If there is a case of injury you may be compensated where appropriate. You can contact the project team at any time by phone: Ryan Avery; (71) 99100-4840, email: ravery3@lsu.edu; Profa. Simone Souza de Oliveira, e-mail: simone23_oliveira@yahoo.com.br and Prof. Aristeu Vieira da Silva, email: aristeuvsilva@uefs.br or by phone (75) 3161-8796 (LAC-UEFS), (75) 3161-8019 (Department of biological sciences). If you have questions related to ethical questions you may call the CEP-UEFS (75) 3161-8067 or contact them via email at cep@uefs.br. The address of the researchers is UEFS-Highway Transnordestina, s/n, Bairro Novo Horizonte – 44,036-900 – Feira de Santana-BA-Department of Biological Sciences – Laboratory of Clinical Analyses – Building LABIO, Room 30.

Feira de Santana – Bahia, ___/___/___

_____

Ryan Avery – Research Project Coordinator
(75) 99100-4840 - LAC (UEFS)(75) 3161-8796

Data from the parent or legal guardian:
Name:_____

Signature: _____

Appendix J. Parental Consent Form in Portuguese

APÊNDICE 4

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (responsável)

Senhor(a),

Estamos realizando uma pesquisa **"Monitoramento e Sistema de Resposta para Geo-Helmintos Usando Modelagem de Nicho Ecológico e Diagnóstico Molecular"** com o objetivo de determinar a prevalência de geohelmintos, infecções parasitárias em pessoas, causadas por Ascaris (Lombriga), Ancylostoma (Amarelão) e Trichuris (Tricuríase) em áreas periféricas urbanas e rurais no município de Feira de Santana, tendo como responsável Dr. Ryan Arvery e colaboração dos professores Drº Aristeu Vieira da Silva e Prof. Drª Simone Souza de Oliveira. Estou convidando o senhor (a) a autorizar como responsável a participação do menor nesta pesquisa, onde serão coletadas amostras de fezes. Este estudo pretende seguir os princípios éticos de pesquisa envolvendo seres humanos, evitando danos/agravos aos participantes e será realizado no Campus da Universidade Estadual de Feira de Santana, Laboratório de Análise Clinicas e parasitológicas (LAC) localizado no LABIO sala 30 que já vem realizando exames de fezes, possuindo estrutura física e de pessoal adequadas para realização dos mesmos. A coleta de fezes será realizada pelo menor ou responsável e para isso vocês receberão previamente um coletor de fezes esterilizado e, a equipe de pesquisadores lhe dará as instruções para a realizar a coleta. Caso o material seja insuficiente ou ocorra qualquer problema na execução do exame, nova coleta poderá ser realizada, devendo o responsável informar e marcar com antecedência o novo procedimento. É importante ressaltar que as técnicas de coleta, armazenamento, transporte e manipulação são executadas com rigoroso critério de biossegurança. Este estudo também pretende estabelecer uma relação entre os resultados obtidos e as condições socioeconômicas, ambientais e sanitárias das áreas estudadas, obtidas durante a entrevista com o chefe da família e/ou responsável, registrada em formulário próprio. As pesquisas envolvendo seres humanos são passíveis de riscos para os sujeitos pesquisados, porém este estudo pode despertar no menor algum tipo de desconforto durante do material biológico. Para tanto, a equipe foi treinada previamente visando minimizar estes ou outros riscos inerentes que possam surgir, bem como a coordenação do estudo e a equipe executora estará à disposição para as possíveis eventualidades e buscará reparar/indenizar prejuízos que ocorrerem por conta da coleta destas amostras e sua utilização nesta pesquisa. A participação do menor é voluntária, se autorizada pelos responsáveis, e a qualquer momento estaremos a sua disposição para qualquer esclarecimento sobre a pesquisa e menor terá todo o direito de desistir dela quando não se sentir devidamente satisfeito (a). Tudo que será perguntado ao menor e todas as suas respostas serão mantidas em segredo assim como os resultados dos exames laboratoriais, e nunca serão apresentadas relacionadas com o nome dele. A entrevista, que será realizada no dia da entrega do material para exame laboratorial em local reservado, será usada apenas neste estudo. Após a pesquisa, os formulários serão guardados sob a responsabilidade do coordenador do estudo por um período de cinco anos e armazenados no LAC-UEFS (LABIO Sala 30), não podendo ser utilizados em nenhuma outra pesquisa. Os materiais biológicos, após os exames especificados no protocolo, serão destinados ao descarte de amostras biológicas segundo os padrões de biossegurança. Os resultados desta pesquisa poderão ajudar a identificar os casos de doenças causadas por parasitos, e com isso propiciar a oportunidade do menor receber os cuidados adequados por parte do serviço de saúde. Quaisquer crianças e jovens menores de 18 anos só participarão com a autorização dos pais ou dos responsáveis, e após assinarem o Termo de Assentimento Livre e Esclarecido. Os resultados também serão utilizados com finalidade científica na produção da Tese de Doutorado do coordenador do projeto, podendo ser apresentados na forma de resumo em eventos científicos, artigos em revistas científicas e relatórios de pesquisa. Após as análises das amostras de fezes, os resultados serão entregues aos responsáveis nos próprios domicílios e as pessoas serão orientadas a procurar a Unidade Básica de Saúde da sua área de abrangência com as recomendações necessárias, caso seja indicado, para que o menor receba assistência adequada por parte do serviço de saúde. Se você autorizar a participação do menor, deverá assinar este termo em duas vias de igual teor. Uma cópia dele ficará com você e a outra com os pesquisadores responsáveis. Por sua colaboração, você não receberá qualquer valor em dinheiro, mas terá a garantia de que todas as despesas necessárias para a realização desta pesquisa não serão de sua

responsabilidade havendo você será compensado. Verificado qualquer dano você poderá ser indenizado se for o caso. Você poderá entrar em contato com a equipe do projeto a qualquer momento que julgar necessário pelos telefones: Ryan Avery; (71) 99100-4840, e-mail: ravery3@lsu.edu; Profa. Simone Souza de Oliveira, e-mail: simone23_oliveira@yahoo.com.br e Prof. Aristeu Vieira da Silva, e-mail: aristeuvsilva@uefs.br ou pelos telefones (75) 3161-8796 (LAC-UEFS), (75) 3161-8019 (Departamento de Ciências Biológicas). Em caso de dúvidas relacionadas a questões éticas você pode ligar para o CEP-UEFS (75) 3161-8067 ou manter contato pelo e-mail cep@uefs.br. Endereço da UEFS – Rodovia Transnordestina, s/n, Bairro Novo Horizonte – 44.036-900 – Feira de Santana – BA - Departamento de Ciências Biológicas – Laboratório de Análises Clinicas - LABIO, Sala 30.

Feira de Santana – Bahia, ___/___/___ _____

Ryan Avery - Coordenador do Projeto de Pesquisa
(75) 99100-4840 - LAC (UEFS)(75) 3161-8796

Dados do pai ou responsável legal:

Nome:_____

Assinatura: _____

Appendix K. Minor Assent Form in English

APPENDIX 5

MINOR TERM OF FREE AND INFORMED CONSENT

You are being invited to participate in research at the State University of Feira de Santana (UEFS). Your parents allowed you to join. We want to know if you have worms like roundworm, hookworm, and whipworm infections and know what can be caused by these parasites. You do not need to participate in research if you don't want to, that's your right, we will have no problem if you quit. The research will be done in neighborhoods and outlying rural areas of Feira de Santana, where we will collect the children's poop. For this, we will use a disposable and sterilized jar to collect your poop. The use of the jar is considered safe. If something wrong happens, you can find the research coordinator Ryan Avery, Profa. Simone Souza de Oliveira and Prof. Aristeu Vieira da Silva anytime by phone: Ryan Avery; (71) 99100-4840, e-mail: ravery3@lsu.edu; Profa. Simone Souza de Oliveira, e-mail: simone23_oliveira@yahoo.com.br and Prof. Aristeu Vieira da Silva, email: aristeuvsilva@uefs.br or by phone (75) 3161-8796 (LAC-UEFS), (75) 3161-8019 (Department of biological sciences). If you have questions related to ethical questions you may call the CEP-UEFS (75) 3161-8067 or get in touch via email at cep@uefs.br. The address of the UEFS researcher team is-Highway Transnordestina, s/n, Bairro Novo Horizonte – 44,036-900 – Feira de Santana-BA-Department of Biological Sciences – Laboratory of clinical analyses – LABIO, 30 Room. The team was trained to minimize risks that may arise, and the study will be coordinated and executed so that the team will be available for any possible issues/problems and will seek to repair any that occur during the poop collection. Study participation is free, you will not receive any money, however all the costs of the examination shall be for covered by the research team. The data will be stored under the responsibility of the study Coordinator for a period of five years and stored in LAC-UEFS (LABIO 30 Room) and may not be used in any other research. If we know that you have a worm(s), we will talk to the doctor and the nurse at the Health Unit, which will help you. Besides the doctor and the nurse at the clinic, no one will know that you are participating in the research, we will not talk to other people, and we will not give strangers the information you give us. The survey results will be published, but without identifying the children who participated in the research. When we finish the research results will be presented to other scientists and professors in research meetings and published in scientific journals. If you have any questions, you can ask me. I wrote down my name and phone number.

Feira de Santana, ____ of _____, _____.

_____ _____

Minor's signature

Ryan Avery
(75) 99100-4840
LAC UEFS-(75) 3161-8796

Appendix L. Minor Assent Form in Portuguese

APÊNDICE 5

TERMO DE ASSENTIMENTO LIVRE E ESCLARECIDO

Você está sendo convidado (a) a participar de uma pesquisa na Universidade Estadual de Feira de Santana (UEFS). Seus pais permitiram que você participe. Queremos saber se você tem verme como lombriga, amarelão e tricuríase e que pode ser causada por estes parasitos. Você não precisa participar da pesquisa se não quiser, é um direito seu, não terá nenhum problema se desistir. A pesquisa será feita em bairros e áreas periféricas e rurais de Feira de Santana, onde será coletado o cocô das crianças. Para isso, será usado um potinho descartável e esterilizado para coletar o seu cocô. O uso do potinho é considerado seguro. Caso aconteça algo errado, você pode procurar o coordenador da pesquisa Ryan Avery, Profa. Simone Souza de Oliveira e Prof. Aristeu Vieira da Silva a qualquer momento pelos telefones: Ryan Avery; (71) 99100-4840, e-mail:ravery3@lsu.edu; Profa. Simone Souza de Oliveira, e-mail: simone23_oliveira@yahoo.com.br e Prof. Aristeu Vieira da Silva, e-mail: aristeuvsilva@uefs.br ou pelos telefones (75) 3161-8796 (LAC-UEFS), (75) 3161-8019 (Departamento de Ciências Biológicas). Em caso de dúvidas relacionadas a questões éticas você pode ligar para o CEP-UEFS (75) 3161-8067 ou manter contato pelo e-mail cep@uefs.br. Endereço da UEFS – Rodovia Transnordestina, s/n, Bairro Novo Horizonte – 44.036-900 – Feira de Santana – BA - Departamento de Ciências Biológicas – Laboratório de Análises Clinicas - LABIO, Sala 30. A equipe foi treinada para minimizar riscos que possam surgir, bem como a coordenação do estudo e a equipe executora estará à disposição para os possíveis danos e buscará reparar prejuízos que ocorrerem por conta da coleta do cocô. A sua participação é de graça, você não receberá nenhum dinheiro, entretanto todas as despesas com o exame serão por conta da pesquisa. Os dados serão guardados sob a responsabilidade do coordenador do estudo por um período de cinco anos e armazenados no LAC-UEFS (LABIO Sala 30), não podendo ser utilizados em nenhuma outra pesquisa. Se soubermos que verme você tem, podemos falar para o médico e para a enfermeira da Unidade de Saúde, que poderão ajudar você. Além do médico e da enfermeira do Posto de Saúde, ninguém saberá que você está participando da pesquisa, não falaremos a outras pessoas, nem daremos a estranhos as informações que você nos der. Os resultados da pesquisa vão ser publicados, mas sem identificar as crianças que participaram da pesquisa. Quando terminarmos a pesquisa os resultados serão apresentados para outros cientistas e professores em reuniões de pesquisa e publicados em revistas científicas. Se você tiver alguma dúvida, você pode me perguntar. Eu escrevi os telefones embaixo do meu nome.

Feira de Santana, ____de _____de _____.

_____	_____
Assinatura do menor	Ryan Avery (75) 99100-4840 LAC UEFS – (75) 3161-8796

Appendix M. Copyright Agreement

Mary Ann Liebert, Inc.
Copyright Transfer Agreement

Article Title: Molecular Confirmation of *Ascaris suum*: Further Investigation into the Zoonotic Origin of Infection in an 8-Year-Old Boy with Loeffler Syndrome
Name of Author: Mr. Ryan Avery
Journal Name: Vector-Borne and Zoonotic Diseases

1. The Contribution
The Author(s) hereby affirm(s):

A. The Contribution entitled "Molecular Confirmation of *Ascaris suum*: Further Investigation into the Zoonotic Origin of Infection in an 8-Year-Old Boy with Loeffler Syndrome" is to be published in Vector-Borne and Zoonotic Diseases.
B. Applicable Supplementary Material shall be published with Contribution in Vector-Borne and Zoonotic Diseases.

2. Obligations of the Authors(s) The Author(s) warrant(s):

A. Contribution and Supplementary Material is original and no part has been plagiarized, fabricated, or manipulated.
B. All individuals identified as Authors* contributed to the Contribution, and all individuals who contributed are listed as Authors on the Contribution. [*See below for definition of "Author."]
C. Contribution is not under consideration in another publication and has not been published elsewhere, or in any other language.
D. Contribution and Supplementary Material contains no libelous or unlawful statements; does not infringe upon the rights (including without limitation the copyright, patent, or trademark rights) or the privacy of others; does not contain material or instructions that might cause harm or injury.
E. If applicable, Contribution meets all ethical guidelines and/or Internal Review Board approval in the treatment of human and animal studies (see Instructions for Authors), or meets the requirements set forth by the Declaration of Helsinki.
F. Names and all identifying features or information have been removed from the Contribution's figures, tables, text, and Supplemental Material.
G. Obtained re-use permission for excerpts from copyrighted works owned by third parties and attribution to the sources have been included in the Contribution.
H. Author(s) have disclosed any conflicts of interest within the Contribution as a paragraph before the reference section.
I. All contributing Authors are aware of the rights and conditions set forth in this agreement.
J. The Authors(s) will indemnify Mary Ann Liebert, Inc., against any costs, expenses, or damages, which may incur, or of which Mary Ann Liebert, Inc., may become liable as a result from any breech of these warranties contained herein. These representations and warranties may be extended to third parties by Mary Ann Liebert, Inc.

Authorship is defined as:

- ***Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND***
- ***Drafting the work or revising it critically for important intellectual content; AND***
- ***Final approval of the version to be published; AND***
- ***Agreement to be accountable for all aspects of the work in ensuring that***

questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

3. Grant of Right

3.1. The Author(s) grant the copyright of this Contribution, including any graphic and/or video elements therein (e.g. illustrations, charts, moving images), is hereby assigned for good and valuable consideration to Mary Ann Liebert, Inc., effective if and when the Contribution is accepted for publication and to the extent assignable if assignability is restricted by applicable law or regulations. If copyright cannot be assigned to Mary Ann Liebert, Inc., because the Author(s) are government employees then the Author(s) agree to inform Mary Ann Liebert, Inc**.**

3.1A. For United States Federal government employees, works created within the scope of their employment are considered to be public domain, and Mary Ann Liebert, Inc., does not require a transfer of copyright for such works. However, Author(s) must sign this agreement and notify Mary Ann Liebert, Inc., of the Contribution's public domain status.

3.1B. For United Kingdom, Canadian, or Australian government employees, works created within the scope of their employment remain under Crown Copyright. This includes material created by civil servants, ministers and government departments, and agencies. The Author(s) agree(s) to grant an exclusive publishing and distribution license to Mary Ann Liebert, Inc.

3.2. The copyright assignment includes without limitation the exclusive, assignable and sub-licensable right, unlimited in time and territory, to reproduce, publish, distribute, transmit, make available and store the Contribution and Supplementary Material, including abstract, in all forms of media of expression now known or developed in the future, including pre- and reprints, translations, photographic reproductions and microform. Mary Ann Liebert, Inc., may use the Contribution and Supplementary Material in whole or in part in electronic form, such as use in databases or data networks for display, print or download to stationary or portable devices. This includes interactive and multimedia use and the right to alter the article to the extent necessary for such use.

4. Retention of Rights

4.1. Author(s) retain the right to self-archive the Author Accepted Manuscript version of their Contribution on their own personal website. Author(s) may also deposit their Author Accepted Manuscript version of the Contribution in any repository, provided it is only made publicly available 12 months after the official publication** or later. The Author(s) may not archive or deposit the final published version (Version of Record), which is published on Liebertpub.com and in the Journal. Furthermore, the Author(s) may only post their Manuscript version provided acknowledgment is given to the original source of publication and a link to the published Contribution in the Journal is inserted. The link must be provided by inserting the Digital Object Identifier (DOI) of the Contribution in the following sentence: "Final publication is available from Mary Ann Liebert, Inc.: http://dx.doi.org/[insert DOI]".

*****For purposes of Clause #4, "Official publication" refers to the final published version (version of record) of the Contribution published within an issue of the Journal.***

4.2. Mary Ann Liebert, Inc., hereby licenses back to the Author(s) the following rights with respect to the final published version (Version of Record) of the Contribution:

4.2A. The right to send or transmit individuals copies of the Contribution to research colleagues upon their specific request provided no fee is charged, and further provided that there is no systematic distribution of the Contribution (e.g. posting on listservs, repository, website or automated delivery). Posting the final published version on the open internet is not permitted.

4.2B. The right to re-use the Contribution or parts thereof for any publication authored or edited by the Author(s) (excluding journal articles) where such re-use material constitutes less than half of the total material in such publication. In such case, any modifications should be accurately noted. Posting the final published version on the open internet is not permitted.

4.2C. The right to include the Contribution in teaching or training duties at the Author(s) institution/employer including course packs, oral presentation, in-house training, and use in theses/dissertations. The Contribution may not be used in seminars outside of normal teaching obligations (e.g. commercial seminars). Posting the final published version on the open internet is not permitted.

5. Reversion of Rights

All rights pursuant to clauses 3 to 4 shall revert to the Author(s) should the Contribution be rejected during the publication process.

6. Open Access

Should the Author(s) purchase open access for the Contribution, before online publication in the Journal, the publishing license granted to Mary Ann Liebert, Inc., in clause 3 will remain exclusive. Mary Ann Liebert, Inc., will apply a Creative Commons open access license to the Contribution and Supplementary Material for the purpose of publication in the Journal on an open access basis. The Author(s) retain(s) the following rights under an open access license: copyright, including patents, trademarks or other intellectual property rights; the right to distribute and reproduce the Contribution for noncommercial use without restriction; and the right to self-archive the Contribution immediately upon publication. For Open Access details, contact OpenAccess@liebertpub.com.

Author Status [choose one]:

I agree to the terms of the agreement and transfer copyright.

Signature: Ryan H. Avery

Date: 23- Jun-2018

Vita

Ryan Harry Avery is from Syracuse, New York, and obtained his undergraduate degree in biology from the State University of New York at Geneseo. Ryan has had a passion for the biological sciences since childhood and decided to make a career of it in high school. He fell in love with parasites during his college years and is glad he found a home to research them in Baton Rouge. He enjoys teaching students, scientific policy, and collaborative international research.